cancer treatment reports

Volume 63
Number 1
January 1979
Pages 1-162

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Arthur C. Upton, *Director*

Division of Cancer Treatment, Vincent T. DeVita, Jr., *Director*

Editorial Board

Bruce A. Chabner, *Editor-in-Chief*
Associate Editors:
Paul A. Bunn
Michael Colvin
Eli J. Glatstein
Marc E. Lippman
John S. Macdonald
Stephen C. Schimpff
Mary K. Wolpert
Daniel S. Zaharko
John L. Ziegler

Editorial Staff

Pamela W. Jones, *Managing Editor*
Jean Griffin Baum, *Assistant Editor*
Elaine P. Beck, *Assistant Editor*
Virginia T. Ascosi, *Editorial Assistant*
Florence L. Barnes
Mary M. Davis
Deidre A. Hoehn

Advisory Board

Bernard Fisher
Emil J Freireich
Lewis W. Guiss
Simon Kramer
Faith H. Kung
LaSalle D. Leffall, Jr.
John A. Montgomery
David G. Nathan
Paul E. Neiman
Stanley E. Order
Theodore L. Phillips
Philip Rubin
Edward F. Scanlon
Stuart F. Schlossman
Joseph V. Simone

Editorial Policy

The Editorial Board will consider manuscripts dealing with all aspects of preclinical and clinical cancer treatment. These may include such topics as surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
3. **Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.**
4. **Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.**
   (a) Ideas relating to cancer treatment.
   (b) Preliminary research reports.
   (c) Clinical case reports.
   (d) Brief clinical trials of novel treatments.

All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $38.35 domestic, with an additional $9.60 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" (the identifying code for this journal) when requesting a subscription.

INSTRUCTIONS TO AUTHORS

Summary
Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double spaced. Each page should begin with a new paragraph. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *Chemical Abstracts* for proper nomenclature.

Abbreviations
Only standard abbreviations are acceptable. If a new or coined abbreviation is used, it must be defined when first mentioned.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Symposia
Requests to publish the proceedings of symposia should be addressed to the Managing Editor. A copy of the agenda and a list of speakers should accompany such requests.

Submission of Manuscripts
Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 8300 Colesville Rd, Rm 3A05, Blair Bldg, Silver Spring, Md 20910. We invite all authors to submit with their manuscripts a short list of possible reviewers. The editorial board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints will be sent to the senior author about three weeks after publication of the article.

Contents

Cancer Treatment Reports
Vol. 63, No. 1
January 1979

Page

ARTICLES

Phase I Study of Thymidine Plus 5-Fluorouracil Infusions in Advanced Colorectal Carcinoma 1
Stanley J. Vogel, Cary A. Presant, Gary A. Ratkin, and Carol Klahr
The Jewish Hospital of St. Louis, Washington University School of Medicine, Missouri

VM–26, a New Anticancer Drug With Effectiveness in Malignant Lymphoma: An Eastern Cooperative Oncology Group Study (EST 1474) 7
Delia F. Chiuten, John M. Bennett, Richard H. Creech, John Glick, Geoffrey Falkson, Harvey S. Brodovsky, Colin B. Begg, Franco M. Muggia, and Paul P. Carbone
Eastern Cooperative Oncology Group

Renal Excretion Kinetics of High-Dose *cis*-Dichlorodiammineplatinum(II) Administered With Hydration and Mannitol Diuresis 13
G. A. Frick, R. Ballentine, C. W. Driever, and W. G. Kramer
College of Pharmacy, University of Houston, Texas

Phase I Trial of Chlorozotocin 17
Richard J. Gralla, Charlotte T. C. Tan, and Charles W. Young
Memorial Sloan-Kettering Cancer Center, New York, New York

Phase II Trial of Adriamycin in the Treatment of Advanced or Recurrent Endometrial Carcinoma: A Gynecologic Oncology Group Study 21
J. Tate Thigpen, H. J. Buchsbaum, C. Mangan, and J. A. Blessing
Gynecologic Oncology Group

***cis*-Dichlorodiammineplatinum(II), Adriamycin, Cyclophosphamide, CCNU, and Vincristine in Non-Small Cell Lung Carcinoma: A Preliminary Report** 29
Hiroshi Takita, Patrick C. Marabella, Francis Edgerton, and Donna Rizzo
Roswell Park Memorial Institute, Buffalo, New York

Animal Models for the Comparative Assessment of Neurotoxicity Following Repeated Administration of Vinca Alkaloids 35
Glen C. Todd, William J. Griffing, William R. Gibson, and Douglas M. Morton
Lilly Research Laboratories, Division of Eli Lilly and Co, Greenfield, Indiana

Cytotoxic Effects of the Lysosomotropic Agents DNA–Ellipticine, DNA–Daunorubicin, and DNA–Ellipticine:Daunorubicin on L1210 Leukemia Cells 43
Richard A. Sorace and Bertrum Sheid
State University of New York, Downstate Medical Center, Brooklyn

Page

Pharmacology and Toxicology of α-2′-Deoxythioguanosine in Cancer Patients 53
G. A. LePage and A. Khaliq
McEachern Laboratory, University of Alberta, Edmonton, Canada

Pharmacokinetic Study of *cis*-Dichlorodiammineplatinum(II) (DDP) in the Beagle Dog: Thermodynamic and Kinetic Behavior of DDP in a Biologic Milieu 59
Andre F. LeRoy, Robert J. Lutz, Robert L. Dedrick, Charles L. Litterst, and Anthony M. Guarino
National Cancer Institute, Bethesda, Maryland

Phase I Evaluation of Piperazinedione in Patients With Advanced Cancer 73
Violante Currie, Thomas Woodcock, Charlotte Tan, Irwin Krakoff, and Charles Young
Memorial Sloan-Kettering Cancer Center, New York, New York

Phase II Study With Sequential L-Asparaginase and Methotrexate in Advanced Refractory Breast Cancer 77
Hwee-Yong Yap, Robert S. Benjamin, George R. Blumenschein, Gabriel N. Hortobagyi, Charles K. Tashima, Aman U. Buzdar, and Gerald P. Bodey
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Enzymatic Assay for the Antitumor Agent *N*-(Phosphonacetyl)-L-aspartic Acid (PALA) 85
Jacqueline Friedman, E. Colleen Moore, Stephen W. Hall, and Ti Li Loo
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Reduction of Daunorubicin Lethality and Myocardial Cellular Alterations by Pretreatment With ICRF–187 in Syrian Golden Hamsters 89
E. Herman, B. Ardalan, C. Bier, V. Waravdekar, and S. Krop
Food and Drug Administration, Washington, DC

Mechanism of the Antimetastatic Action of Dimethyltriazenes 93
G. Sava, T. Giraldi, L. Lassiani, and C. Nisi
Istituto di Farmacologia, Università di Trieste, Italy

Comparison of Various Forms of Therapy in Two Different Mouse Tumor Systems 99
Hub F. J. Dullens, Claus Vennegoor, Roel A. De Weger, Felice Woutersen, Ruud A. Woutersen, and Willem Den Otter
Utrecht State University, The Netherlands

Phase II Study of ICRF–159 in Refractory Metastatic Breast Cancer 111
Richard H. Creech, Paul F. Engstrom, David T. Harris, Robert B. Catalano, and Robert E. Bellet
American Oncologic Hospital, The Fox Chase Cancer Center, Philadelphia, Pennsylvania

Page

Ifosfamide Versus Cyclophosphamide in Combination Drug Therapy for Metastatic Breast Cancer 115
Aman U. Buzdar, Sewa S. Legha, Charles K. Tashima, Hwee-Y. Yap, Gabriel N. Hortobagyi, Evan M. Hersh, George R. Blumenschein, and Gerald P. Bodey
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

BRIEF REPORTS AND PRELIMINARY COMMUNICATIONS

Adriamycin: Comparison of a 5-Week Schedule With a 3-Week Schedule in the Treatment of Breast Cancer 121
Edward W. Knight, John Horton, Thomas Cunningham, Francis Rhie, Stephen Lagakos, Charles Rosenbaum, Samuel Taylor IV, and Juanita Tennant
Baptist Hospital, Pensacola, Florida

New Side Effect of the Hypoxic Cell Sensitizer, Misonidazole 123
Janet Partington, Don Koziol, Don Chapman, Harvey Rabin, and Raul C. Urtasun
University of Alberta and Cross Cancer Institute, Edmonton, Canada

Limited Activity of ICRF–159 in Advanced Acute Leukemia 127
M. T. Bakowski, H. G. Prentice, T. A. Lister, J. S. Malpas, T. J. McElwain, and R. L. Powles
St. Bartholomew's Hospital, London, England

Isotopic Immunoglobulin in an Integrated Multimodal Treatment Program for a Primary Liver Cancer: A Case Report 131
David S. Ettinger, Leon H. Dragon, Jerry Klein, Maggie Sgagias, and Stanley E. Order
The Johns Hopkins Hospital, Baltimore, Maryland

Rubidazone in Metastatic Breast Cancer 135
Sewa S. Legha, Robert S. Benjamin, Aman U. Buzdar, G. N. Hortobagyi, and George R. Blumenschein
The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

Phase II Trial of Hexamethylmelamine in Ovarian Carcinoma Resistant to Alkylating Agents 137
Philip D. Bonomi, John Mladineo, Bonnie Morrin, George Wilbanks, Jr, and Robert E. Slayton
Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Phase II Evaluation of Pyrazofurin in Patients With Advanced Colorectal Carcinoma 139
David S. Carroll, Nancy E. Kemeny, and Richard J. Gralla
Memorial Sloan-Kettering Cancer Center, New York, New York

Page

Phase I–II Study of Hexamethylmelamine Plus Dibromodúlcitol in Refractory Carcinoma of the Breast 141
Albert L. Van Amburg III, Cary A. Presant, Enrique Vélez-García, and Carol Klahr
The Jewish Hospital of St. Louis, Washington University School of Medicine, Missouri

Vincristine Effect on Methotrexate Cerebrospinal Fluid Concentration 143
F. Tejada and C. G. Zubrod
Comprehensive Cancer Center for the State of Florida, Miami

Phase II Study of High-Dose Methotrexate in Patients With Advanced Malignant Melanoma 147
Richard I. Fisher, Bruce A. Chabner, and Charles E. Myers
National Cancer Institute, Bethesda, Maryland

LETTERS

Phase II Study of Nafoxidine in the Therapy for Advanced Renal Carcinoma 149
Lynn G. Feun, Anibal Drelichman, Amnuay Singhakowinta, Vainutis K. Vaitkevicius, and Noboru Oishi
Wayne State University School of Medicine, Detroit, Michigan

Systemic Allergic Reaction to Adriamycin 150
Daniel J. Arnold and Chester T. Stafford
Dwight D. Eisenhower Army Medical Center, Fort Gordon, Georgia

Use of Combination Chemotherapy With CCNU, Bleomycin, and Vincristine in the Treatment of Metastatic Melanoma in Patients Resistant to DTIC Therapy 151
J. D. Everall and P. M. Dowd
The Royal Marsden Hospital, London, England

Nonresponsiveness of Brain Tumors to VM–26 Therapy in Children 155
Margaret P. Sullivan, Jan van Eys, Jay Herson, Kenneth A. Starling, Abdel Ragab, and Charles Sexhauer
Southwest Oncology Group

Combination 6-Mercaptopurine and 6-Methylmercaptopurine Riboside in the Treatment of Adult Acute Leukemia: A Southwest Oncology Group Study 156
James S. Hewlett, Gerald P. Bodey, Henry E. Wilson, and W. J. Stuckey
Southwest Oncology Group

Combination Chemotherapy Pilot Studies in Head and Neck Squamous Cell Cancer 158
Adrian Bianco, Samuel G. Taylor IV, Steven Reich, John M. Merrill, and William D. DeWys
Northwestern University Medical School, Chicago, Illinois

Page

ANNOUNCEMENTS 161

Fourth Annual "Symposium on Advances in Cancer Treatment Research"
Seminars on Oncology at Roswell Park Memorial Institute
Second Advanced Course in Oncology
Psychosocial Care of the Dying Patient
Fellowship in Medical Oncology

The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

Phase I Study of Thymidine Plus 5-Fluorouracil Infusions in Advanced Colorectal Carcinoma [1,2]

Stanley J. Vogel, Cary A. Presant,* Gary A. Ratkin, and Carol Klahr [3]

SUMMARY

In order to determine the minimal toxic dose of a 5-day infusion of 5-fluorouracil (5–FU) in combination with an infusion of thymidine (TdR), 12 patients received TdR at a dose of 8 g/m²/day for 5½ days, beginning at the same time as a 5-day infusion of 5-FU at doses of 5–20 mg/kg/day. Myelosuppression was the dose-limiting toxicity, and the minimal toxic dose of 5–FU was found to be 7.5 mg/kg/day. Gastrointestinal toxicity was minimal to absent. In eight patients with carcinoma of the colon who had received no prior chemotherapy, there were two patients with partial responses (at doses of 5.0 and 7.5 mg/kg/day of 5–FU), two patients with stable disease, one patient with progressive disease, and three patients with early death (two drug-related deaths and one disease-related death). In four patients who had received prior 5–FU, one had stable disease, one had progressive disease, and two had early death (one drug-related death). We conclude that the addition of TdR to 5–FU infusions changes the dose-limiting toxicity from gastrointestinal toxicity to myelosuppression. The minimal toxic dose is decreased to approximately one third of that when 5–FU is administered alone.

[Cancer Treat Rep 63:1–5, 1979]

There has been little progress in the chemotherapy for recurrent or metastatic colorectal carcinoma despite dramatic advances in the treatment of some other disseminated malignancies. 5-Fluorouracil (5–FU) remains the most widely used and studied drug and is the standard to which other agents and combinations of agents are compared. Although the 15%–25% objective response rate with 5–FU alone has been marginally improved by combination with other agents, improvement in survival has been negligible (1).

Some evidence suggests that infusion of 5–FU may produce greater antitumor effects. In a randomized study there was an objective response rate of 44% with 5–FU given by continuous iv infusion for 5 days compared to a response rate of 19% in controls treated with a standard 5-day course by bolus injection (2). However, there was no significant difference in survival. At 5–FU doses of 30 mg/kg/day by infusion for 5 days, the dose-limiting toxicity was severe stomatitis and myelosuppression was minimal. Efforts to confirm the possible improved efficacy of 5–FU by continuous infusions (alone or in combination) have given conflicting results (1,3,4).

Most tumor studies in mice suggest that thymidine (TdR) may improve the therapeutic index of 5–FU. In AKR murine leukemia, 5–FU cytotoxicity as determined by the spleen-colony assay was augmented 100-fold to 1000-fold by TdR administered within 8 hours prior to or within 1 hour after 5–FU (5).[4] However, against L1210 murine leukemia the cytotoxicity of 5–FU was increased only threefold

[1]Received Aug 31, 1978; revised Nov 7, 1978; accepted Nov 27, 1978.

[2]Supported in part by the Multimodality Oncology Project of The Jewish Hospital of St. Louis and Public Health Service grants RR–00036 (Division of Research Resources) and CA–13053 (National Cancer Institute) from the National Institutes of Health, Department of Health, Education, and Welfare.

[3]Division of Hematology-Oncology, The Jewish Hospital of St. Louis, Washington University School of Medicine, Mo.

*_Reprint requests to:_ Cary A. Presant, MD, Division of Hematology-Oncology, The Jewish Hospital of St. Louis, 216 S Kingshighway, St. Louis, Mo 63110.

[4]Santelli G, and Valeriote F. Enhancement of in vitro 5-fluorouracil cytotoxicity by thymidine. Submitted for publication.

with the addition of TdR. Myelosuppression in mice as determined by the spleen-colony assay was increased only 20% with the combination of 5–FU plus TdR compared to 5–FU alone (Santelli G, and Valeriote F, personal communication).

In advanced $CD8F_1$ murine breast carcinoma, weekly therapy with TdR given 30 minutes before 5–FU resulted in a 70% increase in antitumor response, although there was a considerable amount of toxicity with an 11% mortality rate and a 13% incidence of significant weight loss (6,7). Results similar to these were noted in a murine colon tumor. We therefore felt that a clinical trial of TdR plus 5–FU infusion was warranted. We decided to give 5–FU by continuous infusion because of the possibility of better antitumor effects and less toxicity by this schedule. TdR infusion was used since it had previously been shown to be well-tolerated by patients and to produce no toxicity (8). Since experiments on mice at Washington University had shown myelosuppression to be increased by only 20%, we elected to initiate the combination trial at a 5–FU dose that was 50% less than the tolerated dose of 5–FU when infused without TdR.

MATERIALS AND METHODS

Twelve patients with surgically incurable, histologically confirmed gastrointestinal adenocarcinoma were treated. In 11 of these patients, the primary lesion was in the colon or rectum, and in one patient the primary site was unknown. Five patients had developed recurrent disease 7–36 months after initial surgery, and the remainder had metastatic disease at the time of initial diagnosis. Four patients had received prior radiotherapy and four had received prior combination chemotherapy with infusions of 5–FU, CCNU, and vincristine. All patients had measurable lesions. The median age was 60 years (range, 29–74). The Karnofsky performance status was $\geq$ 60% at the start of treatment. All patients gave voluntary informed consent prior to treatment.

5–FU was given by continuous iv infusion for 5 days at doses of 5–20 mg/kg/day. TdR was supplied by the National Cancer Institute, Bethesda, Md (15 g in 0.5 liter of 0.6% NaCl), and was administered by continuous iv infusion at a dose of 8 g/m²/day for 5½ days. The 5–FU and TdR infusions were started simultaneously. Therapy was not initiated unless the platelet count was > 100,000/mm³ and the wbc count was > 4000/mm³. Courses were repeated every 4 weeks until there was evidence of tumor progression or severe toxicity.

The first dose level of 5–FU tested was 15 mg/kg/day (about half of the tolerated dose of 5–FU by infusion without TdR, 25–30 mg/kg/day). When unexpectedly severe myelosuppression was observed in three of six courses at that dosage, the next initial dose level for subsequent patients entered in the study was reduced to 5.0 mg/kg/day.

Dosage adjustments in subsequent courses were made according to observed nadir blood cell counts. Patients who had a nadir granulocyte count of < 500/mm³ or a nadir platelet count of < 25,000/mm³ had a 50% reduction in subsequent 5–FU dose. Patients who had a nadir granulocyte count of > 1500/mm³ and a nadir platelet count of > 75,000/mm³ had an increase in subsequent 5–FU dose to the next level indicated in table 1.

The initial starting dose for subsequent patients entered in the study was increased to the next higher dose level in table 1 only when two or three consecutive patients had not experienced moderate toxicity. Moderate myelosuppression was defined as a nadir granulocyte count of < 1500/mm³ or a nadir platelet count of < 75,000/mm³.

Partial response was defined as a decrease of > 50% in the sum of the products of diameters of bidimensional measurable lesions. In patients with hepatic metastases, partial response was defined as a decrease of > 30% in the sum of the distances between the costal margin and liver edge measured in the midline and right and left midclavicular lines, with no progression of any lesion and no appearance of any new lesions. Stable disease was defined as less than a partial response but without evidence of progression. Progression was defined as the enlargement of an existing lesion by > 25% or the appearance of new lesions.

RESULTS

Twelve patients received a total of 22 courses of 5–FU plus TdR. Myelosuppression was the dose-limiting toxicity, and is summarized in table 1. At 5–FU doses of 5.0–7.5 mg/kg/day there was little myelosuppression. However, in the 14 courses at 5–FU doses of 10–20 mg/kg/day there was frequent, severe myelosuppression and four drug-related deaths. There were four occurrences of nadir granulocyte counts of $\leq$ 100/mm³ and each occurrence was associated with gram-negative septicemia. Three of the instances were observed during the first course of therapy, and the other instance occurred after 6 months of treatment (*see* below). In one instance septicemia in association with ureteral obstruction and urinary tract infection occurred before the onset of granulocytopenia, and was undoubtedly exacerbated by the subsequent myelosuppression. All drug deaths occurred in the four patients with severe granulocytopenia, although in

TABLE 1.—5-FU + TdR infusion (myelosuppression)

5-FU dose (mg/kg/day)	No. of courses	Nadir: Granulocyte count (× 10^3/mm^3)	Nadir: Platelet count (× 10^3/mm^3)	Drug deaths
5.0	2	1.9, 3.7	113, 303	0
7.5	6*	1.5, 2.9, 4.1, 6.0, 6.8	148, 195, 197, 210, 295	0
10.0	5	0.1, 0.1, 1.0, 5.0, 12	31, 49, 83, 112, 350	1
12	2	0, 5.0	35, 370	1
15	6	0, 0, 0.5, 0.6, 7.7, 8.3	0, 1, 3, 57, 430, 530	2
20	1	4.1	72	0

*1 course not evaluable for myelosuppression.

two of the patients the granulocyte counts recovered prior to death. Both of these patients had systemic candidiasis.

Three patients had platelet counts of < 25,000/mm^3, and one patient received prophylactic platelet transfusions. There was no bleeding in any of these three patients. In one patient gastrointestinal bleeding occurred with a platelet count of 35,500/mm^3, but did not contribute to the patient's death. Platelet and granulocyte nadirs occurred 14–19 days after the start of therapy.

The minimal toxic dose of 5–FU in this study appeared to be 7.5 mg/kg/day. At a 5–FU dose of 7.5 mg/kg/day, the mean nadir granulocyte count was 4260/mm^3 and the mean nadir platelet count was 209,000/mm^3.

Myelosuppression was not clearly related to pretreatment blood cell counts, performance status, or organ involvement. The three early drug deaths occurred in patients who had received prior radiotherapy (two patients) and chemotherapy (one patient). One example illustrates toxicity that was apparently related to factors other than 5–FU dosage, possible decreased liver function.

Case Report

A 60-year-old woman developed hepatic metastases 7 months after resection of colon carcinoma (Duke's classification C). She had received no radiotherapy or chemotherapy. Her laboratory studies showed a BUN of 12 mg/dl, an alkaline phosphatase of 243 IU/liter, an SGOT of 22 units/liter, and an LDH of 393 units/liter. She received one course of TdR plus 5–FU at a dose of 10 mg/kg/day, and 4 weeks later she received a second course of TdR plus 5–FU at a dose of 15 mg/kg/day. With the second course, the nadir granulocyte count was 4100/mm^3 and the nadir platelet count was 72,000/mm^3. Because of the patient's desire to remain an outpatient, she then received maintenance therapy with several weekly bolus doses of 5–FU without TdR. After her liver size increased, she received a third course of TdR infusion plus 5–FU at a reduced dose of 10 mg/kg/day. At the start of this course of treatment the neutrophil count was 15,000/mm^3 and the platelet count was 810,000/mm^3. Her laboratory studies showed a BUN of 6 mg/dl, an alkaline phosphatase of 337 IU/liter, an SGOT of 40 units/liter, a bilirubin of 0.7 mg/dl, and an LDH of 715 units/liter. Therapy was complicated by severe neutropenia (50 cells/mm^3), moderate thrombocytopenia (49,000 cells/mm^3), and fatal gram-negative septicemia.

Other toxicity was less frequent and usually occurred at higher doses (table 2). Mild nausea occurred in only one patient. Severe stomatitis was observed in only two instances, both during septicemia. There was mild diarrhea in only two courses.

Of 12 patients treated, five died before re-evaluation for a second course of therapy (table 3). Early death in three patients was drug-related and in two patients was disease-related. One patient who had received prior 5–FU alone had stable disease for 2 months and then progression at 3 months; the other previously treated patient had progressive disease at the 1-month evaluation.

Five patients had received no prior chemotherapy and survived ≥ 4 weeks. One patient with hepatic and pelvic metastases showed a partial response of 7 months' duration after an initial 5–FU dose of 5.0 mg/kg/day. Another patient with hepatic metastases had a partial response of 4+ months' duration fol-

TABLE 2.—5-FU + TdR infusion (other toxic effects)

Toxic effect	No. of occurrences	5-FU dose (mg/kg/day)
Skin rash	1	15
Fever	1	7.5
Stomatitis	3	
Severe	2	15 and 12
Mild	1	15
Diarrhea, mild	2	15 and 20
Nausea, mild	1	7.5

TABLE 3.—Antitumor responses

	No prior 5-FU	Prior 5-FU	Totals
No. of patients	8	4	12
No. of early deaths*	2	1	3
Drug†	2	1	3
Disease	1	1	2
No. of responses‡			
Partial	2 (4+, 7)	—	2
Stable disease	2 (5, 5)	1 (2)	3
Progression	1	1	2
Survival (mos)			
Early death	0.5, 0.5, 1	0.5, 1	
Other	2, 5+, 6, 10, 10+	2+, 4	

* Prior to re-evaluation for 2nd course at 4 wks.

†1 additional patient died of sepsis following drug therapy, but 6 mos after therapy was begun.

‡Duration (in mos) of partial response or stable disease is given in parentheses; early deaths are not included in response tabulation.

lowing an initial 5–FU dose of 7.5 mg/kg/day. Two patients had stable hepatic metastases for 5 months prior to disease progression, and one had progressive disease at 2 months.

DISCUSSION

The addition of TdR to a 5–FU infusion enhanced 5–FU toxicity and changed the type of dose-limiting toxic reaction. The dose-limiting toxicity of 5–FU infusion alone is gastrointestinal with stomatitis and diarrhea (2). In the present study, patients received TdR plus 5–FU at doses of one fourth to one half of those usually employed when administering 5–FU alone by infusion. Ordinarily, such doses would not be expected to produce bone marrow toxicity. Nevertheless, the dose-limiting toxicity in this study was myelosuppression, with fatal granulocytopenia in four patients. Gastrointestinal toxicity was only rarely observed and was mild except for the two occurrences of severe stomatitis with granulocytopenia and sepsis.

A recent report describes similar dose-limiting myelosuppression with 5–FU plus TdR, including a drug-related death in a patient with prior radiotherapy (9). Pharmacokinetic studies in that report indicate that the clearance rate from the plasma of 5–FU when given with TdR was several-fold slower than the clearance rate of 5–FU when given without TdR. The prolongation of 5–FU serum half-life by TdR probably results from competition for the same pyrimidine degradative enzymes (10,11), and may account for at least part of the increased myelosuppression. Assays of 5–FU and metabolites in sera from patients in this study are in progress.

We conclude that in phase II studies of TdR plus 5–FU infusions, TdR may be given at a dose of 8 g/m²/day for 5½ days together with 5–FU at a dose of 7.5 mg/kg/day for 5 days. The dose of 5–FU may be escalated to 10 mg/kg/day if no myelosuppression is observed, but if the hepatic or renal function changes, increased toxicity may be produced. No conclusions can be made regarding antitumor response rates at the present time due to the small number of patients treated.

REFERENCES

1. MOERTEL CG. Chemotherapy of gastrointestinal cancer. Clin Gastroenterol 5:777–793, 1976.
2. SEIFERT P, BAKER HL, REED ML, ET AL. Comparison of continuously infused 5-fluorouracil with bolus injection in treatments of patients with colorectal adenocarcinoma. Cancer 36:123–128, 1975.
3. RATKIN G, PRESANT CA, and ZEFFREN JL. Combination chemotherapy with 5–FU infusion in metastatic colorectal carcinoma. Proc Am Assoc Cancer Res and ASCO 18:322, 1977.
4. GRILLO-LÓPEZ AJ, VÉLEZ-GARCÍA E, and ELLIOTT A. Survival of patients with advanced gastrointestinal cancer treated with 5-fluorouracil (5–FU) drip. Proc Am Assoc Cancer Res and ASCO 18:331, 1977.
5. SANTELLI G. Potentiation of 5-fluorouracil cytotoxicity by thymidine against leukemia cells. Proc Am Assoc Cancer Res and ASCO 18:148, 1977.
6. MARTIN DS, and STOLFI RL. Thymidine (TdR) enhancement of antitumor activity of 5-fluorouracil (FU) against advanced murine ($CD8F_1$) breast carcinoma. Proc Am Assoc Cancer Res and ASCO 18:126, 1977.

7. MARTIN DS, STOLFI RL, and SPIEGELMAN S. Striking augmentation of the in vivo anticancer activity of 5-fluorouracil (FU) by combination with pyrimidine nucleosides: an RNA effect. Proc Am Assoc Cancer Res and ASCO 19:221, 1978.
8. ENSMINGER WD, and FREI E, III. The prevention of methotrexate toxicity by thymidine infusions in humans. Cancer Res 37:1857–1863, 1977.
9. KIRKWOOD JM, and FREI E, III. 5 Fluorouracil (FU) with thymidine (TdR): a phase I study. Proc Am Assoc Cancer Res and ASCO 19:159, 1978.
10. FINK L, CLINE RE, HENDERSON RB, ET AL. Metabolism of thymine (methyl-C^{14} or -2-C^{14}) by rat liver in vitro. J Biol Chem 221:425, 1956.
11. CHAUDHURI NK, MUKHERJEE KL, and HEIDELBERGER C. Studies on fluorinated pyrimidines. VII. The degradative pathway. Biochem Pharmacol 1:328–341, 1958.

VM-26, a New Anticancer Drug With Effectiveness in Malignant Lymphoma: An Eastern Cooperative Oncology Group Study (EST 1474) [1]

Delia F. Chiuten,* John M. Bennett, Richard H. Creech, John Glick, Geoffrey Falkson, Harvey S. Brodovsky, Colin B. Begg, Franco M. Muggia, and Paul P. Carbone [2,3]

SUMMARY

Thirty-six patients with stage III and IV Hodgkin's disease and non-Hodgkin's lymphoma, who had become refractory to conventional chemotherapy, were treated with VM-26. Complete remissions were documented in two patients with diffuse histiocytic lymphoma. Six patients (four with non-Hodgkin's lymphomas and two with Hodgkin's disease) had partial remissions. The overall response rate was 22% (eight of 36 patients). Hematologic toxicity was the most frequent dose-limiting toxicity. Nonhematologic toxic effects were mild and acceptable. This study demonstrates that VM-26 can produce tumor responses in refractory lymphomas. The Eastern Cooperative Oncology Group is currently planning two new phase II studies to incorporate VM-26 with other active new agents, one involving hexamethylmelamine and the other involving *cis*-dichlorodiammineplatinum(II).

[Cancer Treat Rep 63:7–11, 1979]

Podophyllin, the resin of podophyllotoxin, a natural product isolated from the roots of *Podophyllum peltatum* (commonly known as May Apple or Mandrake) (1,2), has been used for the treatment of condyloma acuminata (3,4).

One of its semisynthetic derivatives, VM-26, has been found to have antitumor activity against various transplanted tumors in mice (5,6). The in vitro activity and mode of action of VM-26 have been reviewed (5,7,8). Unlike related spindle poisons, it inhibits DNA synthesis and lyses cells in mitosis.

Early clinical studies with VM-26 have been reviewed by Goldsmith and Carter (9). Responses in man have been reported in bladder cancer (10), brain tumors (11,12), and lymphomas (13–18).

The therapeutic activity of VM-26 in lymphoma was first noted in the initial phase I studies done by Dombernowsky et al (13) and Trempe et al (18). The European studies (14–17) reported significant activity of VM-26 in Hodgkin's disease (HD), reticulum cell sarcoma, and lymphosarcoma, with response rates of 27%–56%, 23%–52%, and 32%–36%, respectively. The initial weekly recommended dose for phase II studies was 67 mg/m^2 (12). Brain tumor patients appear to tolerate higher doses of VM-26 (up to 130 mg/m^2/week) (11). Studies done in Europe using doses of 40 mg/m^2 three times weekly (16) and 30 mg/m^2/day × 5 days (17) induced severe myelo-

[1]Received Sept 5, 1978; accepted Nov 29, 1978.

[2]Supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The following institutions and investigators participated in the study: Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md (*D. F. Chiuten* and *F. M. Muggia*); University of Rochester, NY (*J. M. Bennett; CA-11083*); American Oncologic Hospital, Philadelphia, Pa (*R. H. Creech; CA-18281*); Hospital of the University of Pennsylvania, Philadelphia (*J. Glick; CA-15488*); University of Pretoria, South Africa (*G. Falkson; CA-21692*); Jefferson Medical College, Philadelphia, Pa (*H. S. Brodovsky; CA-14215*); ECOG Statistical Center, Sidney Farber Cancer Center, Boston, Mass (*C. B. Begg; CA-23318*); and ECOG Operations Office, Madison, Wis (*P. P. Carbone; CA-21115*). Other participating institutions were: Georgetown University School of Medicine (*CA-02824*); Tufts University School of Medicine (*CA-07190*); Harbor General Hospital (*CA-21091*); Rush-Presbyterian-St. Luke's Hospital (*CA-10948*); University of Minnesota (*CA-20365*); Pennsylvania Hospital (*CA-13613*); Medical College of Virginia (*CA-10572*); Northwestern Medical Center (*CA-17145*); University of Montreal (*CA-21653*); Baystate Medical Center (*CA-20182*); Chicago Medical School (*CA-14144*); and Ontario Cancer Foundation.

[3]We wish to thank Helen Lever, Rhonda Holmes, and William Soper for their expert technical assistance.

**Reprint requests to:* Delia F. Chiuten, MD, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bldg 37, Rm 6E12, National Institutes of Health, Bethesda, Md 20014.

suppression. Leukopenia has been the most frequent dose-limiting factor (11–19).

VP-16-213, another epipodophyllotoxin analog, has a preclinical spectrum very similar to that of VM-26, although it has some pharmacologic differences (20,21). VM-26 plasma decay kinetics follow a triexponential function with a terminal half-life ranging from 11 to 38 hours, whereas with VP-16-213 there is a biexponential function with a mean terminal half-life of almost 11.5 hours. Clinical studies suggested that this derivative is somewhat better in leukemia (22–25) while VM-26 is more active in lymphoma (14,15,17). A large clinical comparative study of the two derivatives has been completed (25).

In 1974, the Eastern Cooperative Oncology Group (ECOG) launched a phase II study of VM-26 in the treatment of malignant lymphoma. The study was designed to determine the ability of VM-26 to induce and maintain remissions with continued administration.

MATERIALS AND METHODS

Patient selection and assignment of therapy.—Patients with biopsy-proven HD or NHL (non-Hodgkin's lymphoma) whose disease had become resistant to conventional chemotherapy were entered in this study. The Rappaport classification was used in histologic classification, and all slides were reviewed by the National Lymphoma Panel. Tests done in all patients prior to initiation of therapy were: physical examination including tumor measurements, performance status, cbc, platelet and differential counts, urinalysis, chemistry panel and liver enzymes, chest roentgenogram, total serum protein, and electrophoresis. Liver scans were obtained when needed. Pathologic materials, including paraffin blocks when available, were submitted to the designated regional pathologist for review and classification. All patients in the study had measurable disease. Patients were disqualified if they had received previous treatment with myelosuppressive antineoplastic drugs, experimental antitumor agents, or high-dose corticosteroid therapy within 2 weeks (4 weeks for nitrosoureas or radiotherapy) prior to entry into the study. The expected survival was at least 2 months. Adequate renal function, indicated by a BUN of < 25 mg/100 ml, and adequate bone marrow function, indicated by a wbc count of $> 4000/mm^3$ and a platelet count of $> 100,000/mm^3$, were included as additional criteria. Treatment was terminated in the presence of progressive disease on Day 28 or if no change occurred by Day 56.

Patient population.—Thirty-six (nine with HD and 27 with NHL) of 43 patients entered in these studies were evaluable for toxicity and response (table 1). Seven patients were considered unevaluable because of protocol violation, insufficient data from the investigator, and early death. The mean age was 45.9 years (range, 20–73 years). A majority of the patients (72%) had a performance status of 1 or 2 (ECOG scale). All patients had clinically generalized stage III or IV disease and had previously received extensive combination chemotherapy with or without radiotherapy, except for one patient who had radiotherapy alone. Twenty-nine patients (81%) had received cyclophosphamide, vincristine, and prednisone (CVP), while eight (22%) had received mechlorethamine, vincristine, procarbazine, and prednisone. VM-26 was given for an average of 7.5 weeks, with a range of 2.5–39 weeks. Eighty-nine percent of the patients had at least three courses of treatment.

TABLE 1.—VM-26 in malignant lymphoma

Histology*	No. of patients
HL, nodular	1
HL, diffuse	12
WDL, diffuse	3
WDL, nodular	2
PDL, diffuse	2
PDL, nodular	2
PDL, diffuse and nodular	1
Unknown, NHL	4
HD	9
Total	36

*HL = histiocytic lymphoma, WDL = well-differentiated lymphoma, and PDL = poorly differentiated lymphoma.

Drug administration.—The drug was supplied in 5-ml vials containing 50 mg of VM-26, 300 mg of N_2N-demethyl-acetamide, 150 mg of benzyl alcohol, 2500 mg of purified polyethoxylated castor oil, and absolute alcohol (up to 5 ml qs). Because of the hypotensive effects reported with rapid administration (12), the ampuled solution was diluted in 300 ml of 5% dextrose in water and administered over 30 minutes. A weekly dose of 100 mg/m^2 iv was given when the initial wbc count was $> 4000/mm^3$ and the platelet count was $> 100,000/mm^3$. Dose modifications were adjusted according to peripheral counts. Patients with wbc counts $> 4000/mm^3$ and platelet counts $> 100,000/mm^3$ were given the full dose of VM-26. Those patients with wbc counts of between 2500 and $4000/mm^3$ and platelet counts of $> 100,000/mm^3$ or between 50,000 and $100,000/mm^3$ received half the dose. Patients with wbc counts $> 4000/mm^3$ and platelet counts between 50,000 and $100,000/mm^3$ also received half the dose of VM-26. In patients who obtained either complete responses (CRs) or partial responses (PRs) at the end of 56 days, weekly

treatment was continued until disease progression was evident.

Response criteria.—Categories of response were defined as: (*a*) CR, complete regression of all measurable lesions and disappearance of all other objective evidence of lymphoma; (*b*) PR, a decrease of > 50% in the sum of the products of perpendicular diameters of measured lesions; (*c*) progression, any unequivocal appearance of a new lesion persisting for 2 weeks or an increase by 25% of the product of the diameters of any measured tumor over that which was obtained at the onset of the study. The duration of response was measured from date or start of response to documentation of progressive disease.

RESULTS

Therapeutic effects.—Objective responses were observed in six patients (two CRs and four PRs) with NHL and in two patients (PRs) with HD. The histologic types and characteristics of the six responders with NHL are shown in table 2. Four of 12 patients (33%) with diffuse histiocytic lymphoma (DHL) responded to VM-26. Two of the responses were CRs in patients who had not responded to previous chemotherapy. One of the complete responders had progressive disease with liver, nodes, and central nervous system (CNS) involvement, and received intrathecal methotrexate together with VM-26. A CR was documented in this patient with disappearance of disease in the nodes, clearing of CNS leukemia, and normal liver scan lasting for 252 days, which is the longest duration of remission achieved in this study. The other patient who obtained a CR had total disappearance of disease in the nodes lasting for 28 days. A patient with HD, who had dramatic resolution of nodal disease and infiltrative lesions in the left lung, developed marked tumor necrosis with bowel perforation and died of gas gangrene after one course of VM-26. The remaining patients had PRs. The overall response rate was 22% (eight of 36 patients), with a median duration of response of 84 days (range, 21–252 days).

TABLE 2.—Response to VM-26 in NHL

Histology*	Stage	Response to— Prior y	VM-26
DHL	IIIB	No	CR
DHL	IVA	No	CR
DHL	IVB	Yes	PR
DHL	IVB	Yes	PR
WDL, nodular	IIIB	Yes	PR
WDL, nodular	IVA	No	PR

*WDL = well-differentiated lymphoma.

Hematologic effects.—Baseline and weekly blood cell counts were obtained to evaluate hematologic toxicity that was defined as a wbc count of < 3000/mm^3 and a platelet count of < 100,000/mm^3. Twenty-nine patients (81%) experienced significant leukopenia (median, 1500 cells/mm^3; range, 500–2800 cells/mm^3) and/or thrombocytopenia (median, 57,000 cells/mm^3; range, 23,000–98,000 cells/mm^3) (table 3). Both the wbc and platelet count nadirs occurred between Day 7 and 14 and recovered within 1 week. Two patients had documented infections, one with *Salmonella* sepsis and another with a postmortem diagnosis of aspergillosis. There were no major bleeding episodes.

Other toxic effects.—Table 4 shows other toxic effects encountered. Gastrointestinal symptoms, consisting of nausea and vomiting, were generally mild and acceptable. Episodes of diarrhea were reported in two patients. Skin toxicity (17%), fever (6%), and alopecia (3%) occurred occasionally. Three patients had elevation of liver enzymes, but this was attributable to their disease. One patient, who had abdominal involvement (small bowel and mesentery) and marked ascites, developed an elevated BUN of 72 mg/100 ml and bilirubin of 12 mg/100 ml just before death. No hypotension or evidence of cardiovascular symptomatology was encountered in this study as reported in other series (11,12,14,26). There were no deaths related to drug toxicity.

DISCUSSION

In this phase II study, antitumor activity was found in DHL, with 33% of the patients responding in this category. For other histologic subtypes of NHL and HD the numbers are too small for definitive conclusions. The activity of VM-26 in malignant lymphoma is in agreement with reports of previous studies (13–18) and extends observations in one histologic subtype (DHL). The median duration of response of 84 days for all patients who had become refractory to conventional chemotherapy is indicative of the advanced state of these patients and not of drug intolerance. The overall response rate of 22% in the present study is slightly inferior to those noted in European studies (13,14,16,17). This could be due to differences in histologic classification, criteria of responses, and patient population. The trials abroad included a response criteria of < 50% tumor regression, and some of the patients had not undergone any prior treatment. All of the patients in our study had far-advanced disease and, with the exception of one patient, all had undergone previous chemotherapy with or without radiotherapy and

TABLE 3.—Hematologic toxicity in 36 patients receiving VM-26

Hematologic toxicity	No. of patients (%)	Nadir (cells × $10^3/mm^3$)	
		Mean	Range
Leukopenia and/or thrombocytopenia*	29 (81)		
Leukopenia*	21 (58)	1.5	0.5–2.8
Thrombocytopenia†	16 (44)	57	23–98

*Leukopenia = wbcs ≤ $3000/mm^3$.
†Thrombocytopenia = platelets ≤ $100,000/mm^3$.

TABLE 4.—Nonhematologic toxicity of VM-26 in 36 patients

Toxicity	No. of patients	%
Nausea and vomiting	10	28
Diarrhea	2	6
Skin	6	17
Fever*	2	6
Alopecia	1	3

*Chills and fever occurred 10 mins after infusion in 1 patient.

were refractory to this treatment. This study also suggests that there is no cross-resistance between vinca alkaloids and VM-26 since all of the responders had had vinca alkaloids as part of their previous combination chemotherapy.

VM-26 should be considered for future protocols in malignant lymphoma. There is both preclinical and clinical evidence to suggest possible synergism of VM-26 when given in combination with other agents. When VM-26 was given with cytosine arabinoside, Rivera et al (27) reported cures and an increase in lifespan of up to 280% over controls in BDF_1 mice inoculated ip with L1210 ascites tumor cells. This increase in lifespan was higher than when either drug was administered separately. Chauvergne et al (28), in their study in man, reported the additive effect of VM-26 when given with CVP. A higher response was obtained with the four-drug combination. In a separate study, clinical trials using VM-26 in combination with procarbazine and prednisone obtained an overall response rate (CR + PR) of 84% with a median duration of 10 months for complete responders and 6 months for partial responders in stage III and IV HD (29). The two European studies using cyclic VM-26, adriamycin, cyclophosphamide, and prednisone reported response rates of 61% and 85% in NHL (30,31). An ECOG pilot study using VM-26, *cis*-dichlorodiammineplatinum(II), and prednisone showed definite objective responses in three of nine patients (M. O'Connell, personal communication). Further investigation with larger groups of patients using this drug combination is warranted.

To date, it is difficult to select which of the two analogs, VM-26 or VP-16-213, should be advanced for further clinical testing in the absence of comparative data. A large study just completed by the Children's Cancer Study Group (25) suggests that both are active agents, but the experience in malignant lymphoma was rather limited. Nonetheless, there were two PRs using VM-26 and one CR and one PR using VP-16-213 among six patients each treated with these agents. In another recent report, VP-16-213 given at 35 and 45 mg/m^2 daily × 5 had minimal activity as a single agent in heavily pretreated patients with lymphoma (32). No responses were noted in 35 patients with NHL, including 16 with DHL, and only three PRs were seen in 17 patients with HD. Occasional responses to VM-26 after failure with VP-16-213 and vice versa suggest no absolute cross-resistance between the two podophyllotoxin derivatives (19,20,25). Two new phase II studies incorporating VM-26 with either hexamethylmelamine or *cis*-dichlorodiammineplatinum(II) are currently being planned by the ECOG.

REFERENCES

1. KELLY MG, and HARTWELL JL. The biological effects and the chemical composition of podophyllin. A review. J Natl Cancer Inst 14:967–1010, 1954.
2. KELLER-JUSLÉN C, KUHN M, VON WARTBURG A, ET AL. Synthesis and antimitotic activity of glycosidic lignan derivatives related to podophyllotoxin. J Med Chem 14:936–940, 1971.
3. KAPLAN IW. Condylomata acuminata. N Orleans Med Surg J 94:388–390, 1942.
4. KING LS, and SULLIVAN M. The similarity of the effect of podophyllin and colchicine and their use in the treatment of condylomata acuminata. Science 104:244–245, 1946.
5. STÄHELIN H. 4′-Demethyl-epipodophyllotoxin thenylidene glucoside (VM 26), a podophyllum compound with a new mechanism of action. Eur J Cancer 6:303–311, 1970.
6. CATTAN A, POURNY C, CARPENTIER Y, ET AL. Antimetastasis effect of two derivatives of epipodophyllotoxin in mice. Eur J Cancer 12:797–805, 1976.
7. MISRA NC, and ROBERTS D. Inhibition by 4′-demethyl-epipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside)

of human lymphoblast cultures in G_1 phase of the cell cycle. Cancer Res 35:99–105, 1975.
8. KRISHAN A, PAIKA K, and FREI E, III. Cytofluorometric studies on the action of podophyllotoxin and epipodophyllotoxins (VM-26, VP-16-213) on the cell cycle traverse of human lymphoblasts. J Cell Biol 66:521–530, 1975.
9. GOLDSMITH MA, and CARTER SK. 4′-Demethyl-epipodophyllotoxin-β-D-thenylidene glucoside (VM-26)—a brief review. Eur J Cancer 9:477–482, 1973.
10. PAVONE-MACALUSO M, and EORTC GENITO-URINARY TRACT COOPERATIVE GROUP A. Single-drug chemotherapy of bladder cancer with adriamycin, VM-26 or bleomycin. A phase II multicentric, cooperative study. Eur Urol 2:138–141, 1976.
11. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, JR, ET AL. 4′-Demethylepipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasm. Cancer 33:460–467, 1974.
12. MUGGIA FM, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
13. DOMBERNOWSKY P, NISSEN NI, and LARSEN V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
14. MATHÉ G, SCHWARZENBERG L, POUILLART P, ET AL. Two epipodophyllotoxin derivatives, VM-26 and VP 16-213, in the treatment of leukemias, hematosarcomas, and lymphomas. Cancer 34:985–992, 1974.
15. MATHÉ G, SCHWARZENBERG L, POUILLART P, ET AL. Essai de traitement de divers hématosarcomes par le 4′-démethyl-épipodophyllotoxine-β-D-thénylidene glucoside (VM-26 ou EPT). Nouv Presse Med 3:447–451, 1974.
16. SONNTAG RW, SENN HJ, NAGEL G, ET AL. Experience with 4′-demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside); VM-26; NSC-122819 in the treatment of malignant lymphosis. Eur J Cancer 10:93–98, 1974.
17. EUROPEAN ORGANIZATION FOR RESEARCH ON THE TREATMENT OF CANCER. Clinical screening of epipodophyllotoxin VM-26 in malignant lymphomas and solid tumours. Br Med J 2:744–748, 1972.
18. TREMPE G, SYKES M, YOUNG C, ET AL. Phase I trial of the podophyllotoxin derivative VM-26. Proc Am Assoc Cancer Res 11:79, 1970.
19. RIVERA G, AVERY T, and PRATT C. 4-Demethylepipodophyllotoxin 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) and 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213) in childhood cancer: preliminary observations. Cancer Chemother Rep 59:743–749, 1975.
20. ROZENCWEIG M, VON HOFF DD, HENNEY JE, ET AL. VM 26 and VP 16-213: a comparative analysis. Cancer 40:334–342, 1977.
21. ALLEN LM, and CREAVEN PJ. Comparison of the human pharmacokinetics of VM-26 and VP-16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11:697–707, 1975.
22. CAVALLI F, SONNTAG R, and BRUNNER KW. Epipodophyllotoxin VP 16213 in acute non-lymphoblastic leukemia. Br Med J 4:227, 1975.
23. FALKSON G, VAN DYK JJ, VAN EDEN EB, ET AL. A clinical trial of the oral form of 4′-demethyl epipodophyllotoxin-β-D-ethylidene glucoside (NSC 141540) VP 16-213. Cancer 35:1141–1144, 1975.
24. JUNGI WF, and SENN HJ. Clinical study of the new podophyllotoxin derivative, 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213), in solid tumors in man. Cancer Chemother Rep 59:737–742, 1975.
25. BLEYER WA, CHARD RL, JR, KRIVIT W, ET AL. Epipodophyllotoxin therapy of childhood neoplasia: a comparative phase 2 analysis of VM 26 and VP 16-213. Proc Am Assoc Cancer Res and ASCO 19:373, 1978.
26. ROSENSTOCK JG, and DONALDSON MH. Phase I–II trial of VM-26 (NSC-122819) in the treatment of children with late-stage leukemia. Cancer Treat Rep 60:265–267, 1976.
27. RIVERA G, AVERY T, and ROBERTS DW. Response of L1210 to combinations of cytosine arabinoside and VM-26 or VP 16-213. Eur J Cancer 11:639–647, 1975.
28. CHAUVERGNE J, DURAND M, HOERNI B, ET AL. Induction chemotherapy of non-Hodgkin's malignant lymphomas. Preliminary results of a controlled trial. Eur J Cancer 13:399–400, 1977.
29. ECKHARDT S, DOBRENTEY E, and BODROGI I. Results obtained with combination therapy of VM-26, Natulan, and prednisolone in generalized Hodgkin's disease. Chemotherapy 21:248–254, 1975.
30. MISSET JL, POUILLART P, BELPOMME D, ET AL. Combination chemotherapy with adriamycine, VM26, cyclophosphamide and prednisone in lymphosarcoma and reticulosarcoma stage III and IV. Eur J Cancer 13:411–414, 1977.
31. SCHNEIDER M, MANCINI G, FELLA C, ET AL. Synchronization recruitment chemotherapy in non-Hodgkin's hematosarcomas. Eur J Cancer 13:429–431, 1977.
32. CECIL JW, QUAGLIANA JM, COLTMAN CA, ET AL. Evaluation of VP-16-213 in malignant lymphoma and melanoma. Cancer Treat Rep 62:801–803, 1978.

Renal Excretion Kinetics of High-Dose *cis*-Dichlorodiammineplatinum(II) Administered With Hydration and Mannitol Diuresis [1,2]

G. A. Frick, [3,4] R. Ballentine, [3,5] C. W. Driever, [3] and W. G. Kramer [3,6,*]

SUMMARY

The effect of hydration and mannitol diuresis on the nephrotoxicity and renal excretion kinetics of high-dose *cis*-dichlorodiammineplatinum(II) was examined in seven men undergoing treatment for testicular or bladder carcinoma. The elimination half-life averaged 26.8 hours, shorter than values previously reported from studies not employing hydration and diuresis. In addition, no signs of nephrotoxicity were observed. The results suggest that hydration and mannitol diuresis decrease the nephrotoxicity of *cis*-dichlorodiammineplatinum(II) by increasing its rate of elimination.

[Cancer Treat Rep 63:13–16, 1979]

cis-Dichlorodiammineplatinum(II) (*cis*-DDP) is a relatively new chemotherapeutic agent used in the treatment of many human neoplasms, including testicular carcinoma (1). The primary toxic effects encountered with *cis*-DDP are nephrotoxicity, hematopoietic toxicity, and ototoxicity (2,3). Nephrotoxicity is potentially the most serious and is the dose-limiting factor for *cis*-DDP. Currently, high-dose *cis*-DDP (100 mg/m^2) protocols (4)[7] include hydration and mannitol diuresis in an attempt to minimize or prevent renal toxicity.

The purpose of this study was to evaluate the effect of hydration and mannitol diuresis on the renal excretion kinetics of *cis*-DDP. Parameters used for assessing renal toxicity were also monitored to obtain information on the incidence of toxicity in the presence of hydration and mannitol diuresis.

MATERIALS AND METHODS

Patient Selection

Seven male patients undergoing treatment with *cis*-DDP for testicular or bladder carcinoma participated in the study. To qualify, patients were required to have normal renal and hepatic function, as determined by standard laboratory tests, no detectable bone marrow insufficiency from prior radiotherapy or chemotherapy, no known sensitivity to heavy metals, and no known circulatory disorders that would prevent the administration of 6 liters of iv fluid over a 24-hour period. Each patient was informed of the nature of the study and signed an informed consent form.

Two patients were studied twice during consecutive *cis*-DDP treatments. A minimum of 2 weeks elapsed between treatments to allow for wash-out of the previous dose.

Drug Administration and Patient Monitoring

The evening prior to *cis*-DDP administration, the patient was hydrated with 2000 ml of 5% dextrose

[1]Received Sept 11, 1978; revised Nov 13, 1978; accepted Dec 4, 1978.

[2]Presented in part at the 13th Annual ASHP Midyear Clinical Meeting, San Antonio, Tex, Dec 1978.

[3]Department of Clinical Pharmacy (*G. A. Frick, R. Ballentine,* and *C. W. Driever*) and Department of Pharmaceutics (*W. G. Kramer*), College of Pharmacy, University of Houston, Tex.

[4]Veterans' Administration Hospital, Houston, Tex. *Present address:* Pharmacy Services, Veterans' Administration Hospital, Poplar Bluff, Mo.

[5]The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[6]We wish to acknowledge Dr. M. L. Samuels for supplying patients and valuable medical assistance.

[7]Samuels ML. Velban-bleomycin and sequential *cis*-diammine-dichloroplatinum (*cis*-DDP) therapy with mannitol diuresis for advanced testis cancer and extragonadal germinal neoplasia. M2 experimental clinical chemotherapy of solid tumor, registered project No. M2/gm 21.

[*]*Reprint requests to:* Dr. William G. Kramer, Department of Pharmaceutics, University of Houston, Houston, Tex 77004.

in one-fourth normal saline (NS) with 15 mEq of KCl. Fifteen minutes before *cis*-DDP administration, an iv bolus of 50 ml of 25% mannitol was injected. *cis*-DDP was given at a dose of 100 mg/m^2 in 200 ml of NS infused over a 2-hour period. Following the *cis*-DDP infusion, a mannitol solution (40 g/1000 ml of 5% dextrose in one-half NS with 20 mEq of KCl) was infused at a rate of 250 ml/hour for 16 hours.

Complete urine collections were obtained during the *cis*-DDP infusion over 0–1 and 1–2 hours and after the infusion over 0–0.5, 0.5–1, 1–2, 2–4, 4–6, 6–10, 10–14, 14–22, and 22–28 hours. The total volume of each sample was measured and the samples were stored under refrigeration (4°C) until assay.

Prior to and following the *cis*-DDP treatment, the following laboratory values were determined for each patient: platelet and leukocyte counts, blood urea nitrogen, and serum creatinine.

Platinum Assay

Urine samples were analyzed for total platinum using atomic absorption spectrometry (5). Hydrochloric acid and lanthanum chloride were added to each sample to minimize background interference. To account for differences in background due to variation in a patient's urine composition, standards were prepared for each patient using his own blank urine which was collected before *cis*-DDP administration. Calibration curves were linear and the assay was reproducible over the range of 0.5–50 ppm. Each sample was analyzed in triplicate and the values were averaged.

Pharmacokinetic Analysis

Semilogarithmic plots of the rate of urinary platinum excretion against time following the end of the infusion were biphasic, indicative of two-compartment model kinetics. The data were therefore analyzed according to

$$\text{Rate of excretion} = Ae^{-\alpha t} + Be^{-\beta t} \qquad [1]$$

where α is the apparent rate constant for the initial rapid distribution and elimination phase, β is the apparent rate constant for the terminal slow disposition phase, A and B are hybrid constants of the model, and t is the time after the end of the infusion (6). For each set of data, A, α, B, and β were determined by graphic analysis (6); all slopes and intercepts were calculated by linear regression.

The half-life of each exponential phase was calculated according to

$$t^{1}/_{2\alpha,\beta} = 0.693/\alpha,\beta. \qquad [2]$$

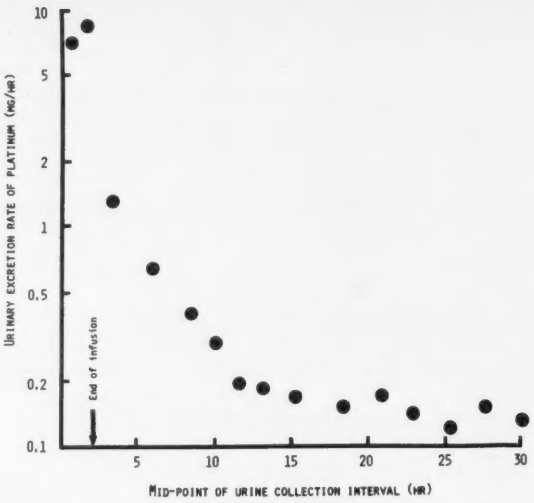


FIGURE 1.—Semilogarithmic plot of rate of urinary excretion of platinum vs time for patient 7a.

RESULTS AND DISCUSSION

A semilogarithmic plot of the rate of urinary excretion of platinum versus time for one patient is shown in figure 1. As noted previously, the curve is biphasic; similar curves were seen for all patients.

Table 1 contains the values for the initial rapid disposition constant, α, the terminal disposition or elimination constant, β, and the apparent half-lives of both phases. Values for α averaged 0.63 hour^{-1} (range, 0.35–1.1 hour^{-1}) and those for β had a mean of 0.032 hour^{-1} (range, 0.012–0.048 hour^{-1}). The elimination half-life, $t^{1}/_{2\beta}$, ranged from 14.4 to 57.7 hours with a mean of 26.8 hours. Over the first 24 hours, an average of 24.0% ± 1.71% of the administered dose of *cis*-DDP was excreted in the urine (table 2).

TABLE 1.—Rapid and slow disposition constants and half-lives for *cis*-DDP

Patient No.	α (hr^{-1})	$t^{1}/_{2\alpha}$ (hr)	β (hr^{-1})	$t^{1}/_{2\beta}$ (hr)
1	0.42	1.65	0.033	21.0
2	0.40	1.73	0.016	43.3
3	0.65	1.07	0.012	57.7
4	0.99	0.70	0.035	19.8
5	1.1	0.63	0.034	20.4
6a	0.70	0.99	0.046	15.1
6b	0.59	1.17	0.043	16.1
7a	0.53	1.98	0.021	33.0
7b	0.45	1.54	0.048	14.4
Mean	0.63	1.27	0.032	26.8
SD	0.27	0.48	0.0132	15.0

TABLE 2.—Comparison of the results of this study with previously reported *cis*-DDP kinetic data

		Ref (No.)					
	This study	DeConti et al (2)	Higby et al (8)			Lange et al (9)	Smith and Taylor (7)
No. of patients/No. of treatments	7/9	4/4	—			2/2	2/2
Dose of *cis*-DDP	100 mg/m²	0.0661–3.15 mg/kg	40–120 mg/m²			2 mg/kg	6 or 7.7 mg (total dose)
Time over which drug was given	2 hrs	1.5 mins	bolus	or	6-hr infusion	—	—
Prehydration	2 liters	None	None			None	None
Posthydration	4 liters	Minimal*	2 liters			None	None
Mannitol	12.5 g before, 40 g/liter × 4 liters after	None	37.5 g in 2 liters after 40 mg of Lasix			None	None
$t^{1}/_{2_\alpha}$	0.63–1.73 hrs	0.4–0.8 hr	—			—	—
$t^{1}/_{2_\beta}$	14.4–57.7 hrs	58.5–73.0 hrs	1.5 hrs† / 1.0 hr‡		12 hrs† / 1.0 hr‡		3–5 days† / 8–10 days‡
% of dose excreted in first 24 hrs	15.4–31.6	18–34	—			30–40 (48 hrs)	25–30
Method	Urine/atomic absorption	Urine/^{193m}Pt	—			^{195m}Pt	^{195m}Pt

*Given only when needed to prevent dehydration from vomiting.
†Half-life based upon plasma level data.
‡Half-life based upon urinary excretion data.

This study of the pharmacokinetics of *cis*-DDP is unique in comparison with previous studies of *cis*-DDP excretion (table 2). This is the first study reported using high-dose *cis*-DDP (100 mg/m²) administered by a 2-hour continuous infusion. It also is unique in that prehydration and mannitol diuresis were administered prior to and immediately following *cis*-DDP infusion.

A summary of the details and results of this study is compared with previous studies in table 2. Such comparisons must be done cautiously and the following factors must be considered: (*a*) dose of *cis*-DDP administered; (*b*) length of time over which the drug was administered; (*c*) extent of patient prehydration; and (*d*) amount of diuresis used. The average elimination half-life of 26.8 hours is shorter than the half-lives reported by DeConti et al (2) which ranged from 58.5 to 73.0 hours or those reported by Smith and Taylor (7) which ranged from 3 to 5 days (plasma data) or from 8 to 10 days (urinary excretion data). Based upon either plasma or urinary excretion data, Higby et al (8) reported a half-life of approximately 1 hour following a bolus injection, but 12 hours from plasma data and 1 hour from urine data following a 6-hour infusion. However, the majority of their data were collected over a shorter period of time and the short half-lives of approximately 1 hour may be more representative of the α phase, which in both our study and that of DeConti et al (2) had essentially a 1-hour half-life. The rate of 24% of the dose excreted in the first 24 hours agrees with the results reported by DeConti et al (2), Lange et al (9), and Smith and Taylor (7).

With one exception, the parameters used to monitor renal function (BUN and serum creatinine) remained normal after each course of *cis*-DDP as compared to pretreatment values (table 3); one patient did experience a transient rise in serum creatinine. The lack of renal problems in this study, as compared to the increased severity of toxic effects seen in studies without prehydration or diuresis (10–13), strongly suggests that prehydration and mannitol diuresis may lessen the nephrotoxicity of *cis*-DDP.

Only one patient experienced side effects other than nausea and vomiting; he experienced some difficulty hearing high tones in the spoken voice range.

A question still unanswered is whether the therapeutic efficacy of *cis*-DDP is decreased as a result of the shorter half-life induced by the prehydration and mannitol diuresis. However, the shorter elimination half-life found in this study may support the hypothesis that hydration and diuresis decrease the nephrotoxicity of *cis*-DDP by increasing its rate of elimination.

TABLE 3.—Laboratory values before/after *cis*-DDP administration*

Patient No.	Platelets (cells × 10^3/mm^3)	Wbcs (cells × 10^3/mm^3)	BUN (mg/100 ml)	Serum creatinine (mg/100 ml)
1	443/302	5.1/6.8	7/3	0.7/0.7
2	310/355	6.0/4.6	9/7	0.7/0.7
3	216/260	5.3/5.2	17/12	0.8/1.1
4	369/313	7.1/6.8	11/11	0.8/0.6
5	293/—	4.2/—	—/—	0.9/—
6a	236/204	5.8/6.0	7/11	0.9/1.1
6b	204/—	6.0/4.6	11/8	1.1/0.8
7a	—/278	—/4.8	20/8	1.1/0.8
7b	263/196	7.8/7.0	12/20	1.1/1.6

*Hospital normal ranges: platelets, 140,000–440,000/mm^3; wbcs, 4000–11,000/mm^3; BUN, 8–20 mg/100 ml; and serum creatinine, 0.5–1.3 mg/100 ml.

REFERENCES

1. EINHORN LH, and DONOHUE JP. *Cis*-diamminedichloroplatinum, vinblastine, and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87:293–298, 1977.
2. DECONTI RC, TOFTNESS BR, LANGE RC, ET AL. Clinical and pharmacological studies with *cis*-diamminedichloroplatinum(II). Cancer Res 33:1310–1315, 1973.
3. GOTTLIEB JA, and DREWINKO B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. Cancer Chemother Rep 59:621–628, 1975.
4. STERNBERG JJ, BRACKEN RB, HANDEL PD, ET AL. Combination chemotherapy (CISCA) for advanced urinary tract carcinoma. JAMA 238:2282–2289, 1977.
5. FRICK GA. Urinary excretion kinetics of platinum in patients following the administration of *cis*-diamminedichloroplatinum(II). Master's Thesis, College of Pharmacy, University of Houston, 1978.
6. GIBALDI M, and PERRIER D. Pharmacokinetics. New York, Marcel Dekker, Inc, 1975, pp 45–88.
7. SMITH PHS, and TAYLOR DM. Distribution and retention of the antitumor agent ^{195m}Pt-*cis*-dichlorodiammine platinum (II) in man. J Nucl Med 15:349–351, 1974.
8. HIGBY DJ, BUCHHOLTZ L, CHARY K, ET AL. Kinetics of *cis*-platinum (DDP) with intensive diuresis. Proc Am Assoc Cancer Res and ASCO 18:110, 1977.
9. LANGE RC, SPENCER RP, and HARDER HC. The antitumor agent *cis*-$Pt(NH_3)_2Cl_2$: distribution studies and dose calculation for ^{193m}Pt and ^{195m}Pt. J Nucl Med 14:191–195, 1973.
10. HIGBY DJ, WALLACE HJ, JR, ALBERT DJ, ET AL. Diamminodichloroplatinum: a phase I study showing responses in testicular and other tumors. Cancer 33:1219–1225, 1974.
11. HILL JM, LOEB E, MACLELLAN A, ET AL. Clinical studies of platinum coordination compounds in the treatment of various malignant diseases. Cancer Chemother Rep 59:647–659, 1975.
12. TALLEY RW, O'BRYAN RM, GUTTERMAN JU, ET AL. Clinical evaluation of toxic effects of *cis*-diamminedichloroplatinum (NSC-119875)—phase I clinical study. Cancer Chemother Rep 57:465–471, 1973.
13. YAGODA A, WATSON RC, GONZALEZ-VITALE JC, ET AL. *Cis*-dichlorodiammineplatinum(II) in advanced bladder cancer. Cancer Treat Rep 60:917–923, 1976.

Phase I Trial of Chlorozotocin [1,2]

Richard J. Gralla,* Charlotte T. C. Tan, and Charles W. Young [3,4]

SUMMARY

Chlorozotocin was given to 37 patients with advanced malignant tumors in a daily × 5 schedule at 6-week intervals. Total iv doses for each course ranged from 75 to 200 mg/m². Myelosuppression was dose-limiting, with a platelet count depression regularly observed at doses of ≥ 150 mg/m²; leukopenia occurred only at the highest dose level. Nausea and vomiting were mild and uncommon. No hyperglycemia or adverse drug-related effects on renal or hepatic function were observed. No major antitumor activity occurred; however, three patients with renal cell carcinoma and one patient each with lung cancer, ovarian carcinoma, and Hodgkin's disease had minor objective decreases in tumor size. A dose range of 150–200 mg/m² iv for each 5-day course is recommended for phase II studies.

[Cancer Treat Rep 63:17–20, 1979]

Chlorozotocin is a water-soluble nitrosourea which shares structural features (fig 1) with both streptozotocin and the chloroethyl nitrosoureas (BCNU, CCNU, and methyl-CCNU). It has been proposed that the myelosuppression produced by nitrosoureas correlates with their relative carbamoylating activities and that their alkylating activities are responsible for antitumor effects (1). Structure-activity relationships suggest that the aminoglucose carrier and the low carbamoylating activity of streptozotocin may contribute to its low bone marrow toxicity. Chlorozotocin, which differs structurally from streptozotocin only by the substitution of a chloroethyl group for the methyl group, has demonstrated high alkylating (2) and low carbamoylating (3) activities. Interest in chlorozotocin, therefore, relates to its potential for improvement in the therapeutic index over that of currently used nitrosoureas.

Chlorozotocin produced long-term survival in L1210 leukemia, P388 leukemia, and B16 melanoma but was only minimally active against Lewis lung carcinoma.[5] At the maximum nonlethal dose of 15 mg/kg ip in the L1210 leukemia system, chlorozotocin was found to produce only minimal depression of peripheral wbc counts and no significant reduction in bone marrow DNA synthesis as measured by ^{3}H-thymidine incorporation. Both chlorozotocin at a dose of 15 mg/kg ip and BCNU at a dose of 30 mg/kg ip resulted in an equal inhibition of L1210 cell DNA synthesis (94%) at 48 hours, but the latter drug reduced bone marrow DNA incorporation of thymidine to 37% of that in control animals (4).

In a completed clinical trial of chlorozotocin given as a single iv injection every 6 weeks at dose levels of 5–150 mg/m², no bone marrow, hepatic, or renal toxic effects occurred in the 27 patients entered in the study (5). At the highest dose level, one patient with nodular lymphocytic lymphoma had a partial response (PR). The present communication reports the results of a phase I trial of chlorozotocin utiliz-

[1] Received May 5, 1978; revised Nov 3, 1978; accepted Nov 14, 1978.

[2] Supported in part by contract N01-CM-43804 (Division of Cancer Treatment) and Public Health Service grants CA-05826 and CA-08748 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] Clinical Chemotherapy Service, Department of Medicine (*R. J. Gralla* and *C. W. Young*) and Department of Pediatrics (*C. T. C. Tan*), Memorial Sloan-Kettering Cancer Center, New York, NY.

[4] We wish to thank Kathleen Trainor for preparation of the manuscript and Laura Lapp for assistance with analysis of the data.

* *Reprint requests to:* Dr. Richard J. Gralla, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

[5] Helman LJ, and Slavik M. Clinical brochure: chlorozotocin (NSC-178248). Prepared by the National Cancer Institute, Bethesda, Md, 1976.

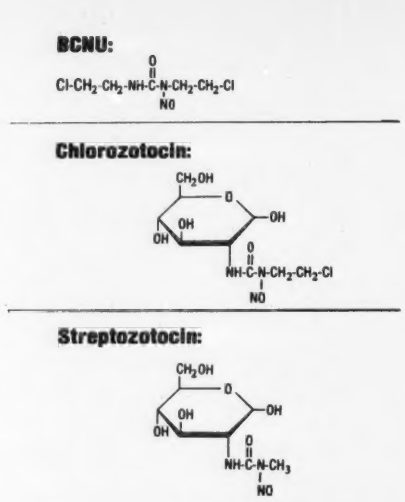


FIGURE 1.—Structural formulas of BCNU, chlorozotocin, and streptozotocin.

ing a 5-day administration schedule carried to a higher maximum dose level.

MATERIALS AND METHODS

Patients with histologic evidence of advanced malignancy for whom standard therapy was not indicated were included in this study. Each patient had a performance status ≥ 50 (Karnofsky scale) and an estimated survival of at least 8 weeks. No patient had received other therapy for at least 30 days prior to receiving chlorozotocin. All patients had acceptable hematologic status (wbc count ≥ 4000/mm³ and platelet count ≥ 120,000/mm³) as well as normal hepatic and renal function. Although not required for inclusion in this phase I trial, all 37 patients had objectively measurable disease.

Chlorozotocin was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md) in sterile vials containing 50 mg of lyophilized powder and was dissolved in sterile water for injection (without preservative) to a concentration of 10 mg/ml immediately before administration. The drug was given daily by rapid iv injection in equal doses for 5 days. Courses were repeated every 6 weeks if blood cell counts were in the normal range or were delayed until blood cell counts returned to normal levels. This trial was initiated at a total dose level of 75 mg/m². In the absence of dose-limiting toxicity, increases in dose level were carried out at increments of 25 mg/m² for each 5-day course, until a total dose of 200 mg/m² (40 mg/m²/day) was reached.

Prior to the start of chemotherapy, all patients had a complete history and physical examination and laboratory evaluation including: cbc with differential count, 12-channel screening profile, 5′-nucleotidase, serum electrolytes and glucose, prothrombin time, serum creatinine, urinalysis, electrocardiogram, and chest roentgenogram. Weekly followup studies included all of the above except the electrocardiogram and chest roentgenogram which were repeated monthly.

TABLE 1.—Characteristics of 31 patients treated with chlorozotocin

Characteristic	No. of patients
Age (range, 18–72 yrs; median, 57 yrs)	
Performance status (Karnofsky scale)	
50–60	9
70–90	22
Prior treatment	
Chemotherapy only	6
Radiation therapy only	4
Radiation therapy and chemotherapy	14
No prior treatment	7

Response criteria were defined as: *PR*—a > 50% decrease of all measurable disease for ≥ 1 month; *minor response*—an objective (≥ 25%) tumor decrease, but less than that required for PR; *stabilization*—a < 25% change in tumor size; and *progression*—an objective increase in measurable disease or the appearance of new tumor lesions.

Some characteristics of the patient population are outlined in table 1 and the various tumor types of these patients are listed in table 2.

RESULTS

Myelosuppression proved to be the dose-limiting toxic effect in this phase I trial. As outlined in table 3, thrombocytopenia was the most frequently observed hematologic effect of chlorozotocin. While some patients had thrombocytopenia at lower dose levels, median platelet count nadirs of < 120,000/mm³ did not occur until the total dose per course exceeded 125 mg/m². One third of the courses (four of 12) at a dose of 200 mg/m² resulted in platelet count depression to ≤ 30,000/mm³, and two patients required platelet transfusion for minor but persistent hemorrhage. Blood cell count depression was generally maximal at 4–5 weeks after initiation of chlorozotocin; at the higher dose levels, the platelet count nadir occurred a few days prior to the maximum leukopenia. In addition, recovery of platelet counts to normal levels was observed earlier (median, Day 38; range, Days 29–57) and occurred more rapidly than recovery of wbc counts (median, Day 46; range, Days 36–59).

TABLE 2.—Responses of 31 patients treated with chlorozotocin

Tumor type	No. of patients evaluable	No. of patients with— PR	Minor regression	Stable disease	Progression
Lung carcinoma*	11	0	1	1	9
Renal cell carcinoma	10	0	3	1	6
Sarcoma†	3	0	0	0	3
Ovarian carcinoma	2	0	1	1	0
Hodgkin's disease	2	0	1	0	1
Islet cell carcinoma of pancreas	1	0	0	0	1
Adenoid cystic carcinoma of parotid	1	0	0	1	0
Neuroblastoma	1	0	0	1	0
Totals	31	0	6	5	20

*7 adenocarcinomas and 4 epidermoid carcinomas.
†2 synovial sarcomas and 1 embryonal rhabdomyosarcoma.

TABLE 3.—Chlorozotocin toxicity in 31 patients

Total dose (mg/m²/ 5-day course)*	No. of patients evaluable	No. of courses	Platelet count nadir Median (cells × 10^3/mm³)	Range (cells × 10^3/mm³)	Median day	Wbc count nadir Median (cells × 10^3/mm³)	Range (cells × 10^3/mm³)	Median day	Median hemoglobin fall (g/100 ml)	No. of patients with— Nausea	Vomiting	Skin rash
75	8	9	160	(25–326)	28	4.4	(3.4–1.3)	19	1.1	3	1	1
100	4	4	169	(14–237)	27	4.3	(1.4–6.4)	24	2.5	0	0	0
125	8	8	160	(59–185)	26	5.8	(1.6–9.8)	26	0.9	2	2	0
150	8	8	117	(29–208)	28	5.1	(3.6–9.7)	23	0.7	0	0	0
175	11	13	84	(16–174)	36	4.3	(2.9–6.3)	43	2.0	1	1	0
200	9	12	51	(15–192)	32	3.8	(1.2–10.8)	36	1.3	1	0	0

* Drug administered in 5 equal daily doses.

Leukopenia occurred less frequently than thrombocytopenia. A median wbc count suppression of < 4000/mm³ was observed only at a dose of 200 mg/m²/course. There was only one instance of leukopenia resulting in septicemia in the 54 courses of chlorozotocin. Bone marrow examination in five leukopenic patients revealed generalized hypoplasia with suppression of all marrow elements (with a decrease in myeloid:erythroid ratio) in each patient and the presence of metastatic tumor in one patient with renal cell carcinoma. Anemia related to chlorozotocin was an uncommon finding; the median fall in hemoglobin ranged from 0.7 to 2.0 g/100 ml at the higher dose levels.

Acute side effects of chlorozotocin were generally mild and infrequent in patients receiving the drug over a 5-day period. Only seven of 54 courses were accompanied by nausea or vomiting, which was mild in all but one instance. The nausea occurred from 2 to 16 hours after drug administration, was of brief duration, and was well controlled by oral phenothiazines when necessary. One half of the patients reported mild anorexia which resolved after the 5-day administration period ended. There were no instances of phlebitis or skin ulceration at injection sites. One patient (treated at 75 mg/m²) with normal platelet counts and without prior dermatologic complaints experienced a generalized petechial rash 48 hours after initiation of her second course of chlorozotocin. A skin biopsy revealed a vasculitis with lymphocyte infiltration around the capillaries. Chlorozotocin was withheld and the rash cleared within 4 days. On rechallenge 1 month later at the same dose level there was no recurrence of the rash.

No patient in this trial experienced hepatic dysfunction, azotemia, or hyperglycemia associated with chlorozotocin, as determined by serial SGOT, bilirubin, 5'-nucleotidase, alkaline phosphatase, BUN, serum creatinine, and serum glucose determinations.

Of the 37 patients enlisted in this trial, 31 were

evaluable for toxic effects through 54 courses of chlorozotocin. Four patients were considered inadequately treated due to early death (31, 30, 15, and 15 days after initiation of treatment), one patient refused completion of the 5-day course of drug, and there was one instance of protocol violation.

Antitumor activity occurred in six of the 31 evaluable patients. In all cases (table 2) only minor responses lasting from 4 to 6 weeks were observed. These responses were manifested by shrinkage of pulmonary nodules in three patients with renal cell carcinoma, in one patient with adenocarcinoma of the lung, and in one patient with stage IV nodular sclerosing Hodgkin's disease. One patient with ovarian carcinoma experienced transient shrinkage of lymphadenopathy. Of these six patients, two had not received prior chemotherapy or radiotherapy (both with renal cell carcinoma), one had only been treated with radiotherapy (patient with adenocarcinoma of the lung), and the other three had been treated with both modalities (including a nitrosourea in the patient with Hodgkin's disease).

DISCUSSION

Clinical investigation of chlorozotocin has been undertaken to determine if this water-soluble nitrosourea with demonstrated high alkylating and low carbamoylating activities in experimental systems (2) would have decreased myelosuppression. Although initial laboratory observations have supported this view (2,4), Anderson et al have cautioned that the bone marrow sparing feature of chlorozotocin is relative and that higher doses in mice produced predictable myelosuppression (4). In this present study, chlorozotocin produced consistent median platelet count depression when doses of $\geq$ 150 mg/m^2/5-day course were given. Higher levels (175 and 200 mg/m^2) produced a linear decrease in the median nadirs of platelet counts, but a lesser effect was noted on leukocyte depression. Milligram for milligram the thrombocytopenia of chlorozotocin appears to be similar to that experienced with BCNU (6,7); however, on a molar basis, chlorozotocin (molecular weight 314 versus 214 for BCNU) may produce greater platelet count depression.

While it is possible that the drug administration in divided doses over 5 days produced greater myelosuppression than a single injection, Kemeny et al (8) observed less thrombocytopenia with divided doses of methyl-CCNU in a randomized trial compared to single-dose methyl-CCNU, with both schedules given in combination with vincristine and 5-fluorouracil.

Patients receiving higher doses of chlorozotocin were in good physiologic condition, with a median performance status of 70; only eight of 20 had received prior radiation therapy and only six of 20 had been previously treated with a nitrosourea or chlorozotocin at lower dose levels. However, of those eight patients experiencing platelet counts < 30,000/mm^3, all had received prior chemotherapy. None of the 11 patients without previous chemotherapy had thrombocytopenia of this magnitude. Cumulative myelosuppression was not noted; however, only four patients received the drug for three or more cycles at the higher dose levels and a definite conclusion on this point cannot be made at present.

The purpose of this study was to define the dose of chlorozotocin that would produce acceptable toxicity in order to conduct disease-oriented trials. With the acceptability and manageability of the observed toxicity, we suggest a phase II administration schedule of 200 mg/m^2 iv divided over 5 days for good-risk patients, 175 mg/m^2 iv over 5 days for those patients who are previously treated, and 150 mg/m^2 iv given over 5 days for those patients who have received heavy pretreatment or have renal or hepatic abnormalities.

REFERENCES

1. WHEELER GP, BOWDON BJ, GRIMSLEY JA, ET AL. Interrelationships of some chemical, physiochemical, and biological activities of several 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 34:194–200, 1974.
2. PANASCI L, GREEN D, and SCHEIN P. Chlorozotocin (CLZ): mechanism of reduced myelotoxicity for mice. Proc Am Assoc Cancer Res and ASCO 18:123, 1977.
3. SCHEIN PS, PANASCI L, WOOLLEY PV, ET AL. Pharmacology of chlorozotocin (NSC-178248), a new nitrosourea antitumor agent. Cancer Treat Rep 60:801–805, 1976.
4. ANDERSON T, MCMENAMIN MG, and SCHEIN PS. Chlorozotocin, 2-[3-(2-chloroethyl)-3-nitrosoureido]-D-glucopyranose, an antitumor agent with modified bone marrow toxicity. Cancer Res 35:761–765, 1975.
5. HOTH D, SCHEIN P, MACDONALD J, ET AL. Phase I trial and clinical pharmacology of chlorozotocin (CLZ). Proc Am Assoc Cancer Res and ASCO 18:309, 1977.
6. WASSERMAN TH. The nitrosoureas: an outline of clinical schedules and toxic effects. Cancer Treat Rep 60:709–711, 1976.
7. DEVITA VT, CARBONE PP, OWENS AH, JR, ET AL. Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea, NSC-409962. Cancer Res 25:1876–1881, 1965.
8. KEMENY N, YAGODA A, and GOLBEY R. Randomization study of 2 different schedules of methyl CCNU (MeCCNU), 5-fluorouracil (5-FU), and vincristine (VCR) for metastatic colorectal carcinoma. Proc Am Assoc Cancer Res and ASCO 18:336, 1977.

Phase II Trial of Adriamycin in the Treatment of Advanced or Recurrent Endometrial Carcinoma: A Gynecologic Oncology Group Study [1,2]

J. Tate Thigpen, H. J. Buchsbaum, C. Mangan, and J. A. Blessing [3,*]

SUMMARY

Forty-three patients with advanced or recurrent endometrial carcinoma no longer amenable to management with surgery or radiotherapy were treated with adriamycin. Sixteen of the 43 patients demonstrated objective response to drug therapy with a ≥ 50% reduction in the size of measurable disease. There were 11 complete responses among these 16 responders. Responders had a significantly better survival than nonresponders ($P < 0.05$). Initial performance status was the only factor of demonstrable prognostic significance. Toxicity was similar to that observed in other phase II trials of adriamycin. Adriamycin, based on these data, is an active agent in the treatment of advanced or recurrent endometrial carcinoma.

[Cancer Treat Rep 63:21–27, 1979]

Endometrial carcinoma is one of the most common gynecologic cancers. Because of the early occurrence of dysfunctional bleeding as a sign of the presence of the neoplasm as well as the high proportion of cases which are well-differentiated (grade I) lesions, most of the cases are diagnosed at an early stage and are cured by surgery alone or surgery plus radiotherapy. For those patients with more advanced disease, however, the prognosis is poor. The literature is replete with reports on the treatment of early disease although many studies are neither well-controlled nor well-designed. Very little data, on the other hand, are available on the therapy for advanced or recurrent endometrial carcinoma. Available information establishes the usefulness, although not the precise level of efficacy, of progestational agents (1–3). Little data are available regarding the use of cytotoxic drugs; those which are available have been compiled from a variety of sources (4). The current study was undertaken by the Gynecologic Oncology Group (GOG) to establish the efficacy of the cytotoxic agent adriamycin in the treatment of advanced or recurrent endometrial carcinoma.

MATERIALS AND METHODS

Between February 1, 1975, and December 31, 1976, member institutions of the GOG entered 55 patients with advanced or recurrent endometrial carcinoma in a study which required treatment with adriamycin. Patients entered in the study had to have (*a*) histologically confirmed endometrial carcinoma no longer amenable to surgical or ra-

[1]Received June 23, 1978; revised Oct 25, 1978; accepted Nov 13, 1978.

[2]Supported by the following Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare: CA-10378 (Gynecologic Oncology Group), CA-19189 (Gynecologic Oncology Group, Walter Reed Army Hospital, and in part the University of Indiana, the University of Utah Medical Center, the University of North Carolina, and the University of Texas at Galveston), CA-13633 (University of Mississippi Medical Center), CA-19502 (University of Iowa), CA-15977 (Hospital of the University of Pennsylvania), CA-13641 (University of Southern California at Los Angeles), CA-12483 (New York Medical College), CA-12478 (Hahnemann Medical College-Jefferson Medical College), CA-12482 (University of Rochester), CA-12477 (Wayne State University), CA-12484 (University of Alabama), and CA-12476 (State University of New York at Buffalo). Other unfunded participants in the study were: Temple University, Rhode Island Hospital, and University of Syracuse.

[3]Department of Medicine, University of Mississippi, Jackson (*J. T. Thigpen*), Division of Gynecologic Oncology, University of Iowa, Iowa City (*H. J. Buchsbaum*), Department of Obstetrics and Gynecology, Hospital of the University of Pennsylvania, Philadelphia (*C. Mangan*), and Group Statistician, Gynecologic Oncology Group, Roswell Park Memorial Institute, Buffalo, NY (*J. A. Blessing*).

**Reprint requests to:* Gynecologic Oncology Group Headquarters, PO Box 60, Philadelphia, Pa 19150.

diotherapeutic management and no longer responsive to hormonal therapy, and (*b*) measurable disease, adequate blood cell counts (granulocyte count $> 2000/\mu l$ and platelet count $> 100,000/\mu l$), normal BUN and creatinine, no evidence of congestive heart failure (CHF), and no prior therapy with adriamycin. Patients entered were also required to have received no previous therapy for at least 3 weeks. Of these 55 patients, seven were deemed ineligible for the study (two patients had no measurable disease, two had incorrect diagnoses, one died prior to therapy, one had a second primary at another site, and one had unacceptable concomitant therapy). An additional three patients refused therapy after one dose of drug and permitted no followup evaluation of tumor response or toxicity. In two other patients, inadequate data are available to permit evaluation of response or toxicity. A final total of 43 patients are available for analysis. The 43 patients each received adriamycin at a dose of 60 mg/m^2 iv every 3 weeks. The initial drug dose was reduced by 50% if the total bilirubin was 1.1–3.0 mg/100 μl or if the alkaline phosphatase was elevated. If no adverse effects of moderate or greater severity were seen, subsequent doses were escalated by 25% to a maximum single dose of 60 mg/m^2. Patients with a total bilirubin of > 3.0 mg/100 μl were not eligible for the study. Subsequent doses were reduced by 25% if the nadir granulocyte count was $< 500/\mu l$ or if the nadir platelet count was $< 50,000/\mu l$. No subsequent course was started unless the granulocyte count was $> 1500/\mu l$ and the platelet count was $> 100,000/\mu l$. The drug was permanently discontinued if CHF developed or if the total cumulative dose reached 500 mg/m^2.

Each of these 43 patients had a histologically confirmed diagnosis of endometrial carcinoma and had received no prior therapy with adriamycin. In all patients previous therapy had been discontinued for a minimum of 3 weeks prior to entry in the study and all had measurable evidence of endometrial carcinoma. The pre-study granulocyte count was $> 2000/\mu l$ and the platelet count was $> 100,000/\mu l$. Renal and hepatic functions were normal in each patient as documented by BUN, creatinine, SGOT, alkaline phosphatase, and bilirubin. With respect to the cardiovascular system, CHF was the only absolute contraindication; despite this, one of the 43 patients did have CHF at the time of entry in the study but did not die from cardiac problems. The present study represents a fair trial of the efficacy of adriamycin since 33 of the 43 patients had a performance status (PS) of 0 or 1 (table 1) and only two of the 43 patients had received prior cytotoxic drug therapy.

TABLE 1.—PS scale used by the GOG

PS	Description
0	Fully active
1	Restricted from physically strenuous activity but ambulatory
2	Ambulatory, capable of self-care, unable to work, up 50% of waking hours
3	Limited self-care, confined to bed or chair ≥ 50% of waking hours
4	Completely disabled, no self-care

Criteria used to gauge response and toxicity and to quantitate PS are reasonably standard among the cooperative oncology groups. Response was gauged by objective measurement of documented disease. A complete response (CR) was defined as the disappearance of all gross evidence of disease for ≥ 1 month. A partial response (PR) was defined as a ≥ 50% reduction in the products obtained from measurement of perpendicular diameters of each lesion with a duration of reduction of ≥ 1 month. Progressive disease (PD) was defined as a ≥ 50% increase in the product obtained from measurement of perpendicular diameters of any lesion or the appearance of any new lesion. Stable disease (SD) was defined as disease not meeting any of the above three criteria. Survival was defined as the observed length of life from entry in the study to death or, for living patients, the date of last contact. Progression-free interval (PFI) was defined as the time from entry in the study to the date of reappearance of, or documentation of increasing parameters of, disease or to date of last contact. This category was used for patients with SD or PD. Response duration was defined as the time from documentation of objective response to date of reappearance of, or documentation of increasing parameters of, disease or to date of last contact.

With regard to adverse effects, hematologic toxicity was graded as shown in table 2. Other adverse effects are listed as mild, moderate, or severe, or are described fully.

RESULTS

Response

The response to therapy of the 43 evaluable patients is presented in table 3. Only 32.6% of the patients demonstrated continued increase of disease after receiving therapy, whereas 37.2% responded with a ≥ 50% reduction in tumor volume. Even more striking is the fact that 25.6% of the patients entered CR. All of the patients who received at least

TABLE 2.—Hematologic toxicity grading*

Grade	Leukocyte count/μl	Granulocyte count/μl	Platelet count/μl
0	≥ 4000	≥ 1500	≥ 150,000
1	3000–3999	1000–1499	100,000–149,999
2	2000–2999	500–999	50,000–99,999
3	1000–1999	250–499	250,000–49,999
4	< 1000	< 250	< 25,000

*Hematologic toxicity was graded in a standard fashion.

TABLE 3.—Objective responses to adriamycin*

Response	No. of patients	%
CR	11	25.6
PR	5	11.6
SD	13	30.2
PD	14	32.6
Total	43	100

*Objective responses to adriamycin were observed in 37.2% of patients with an additional 30.2% demonstrating SD.

a single dose of drug and followup tumor measurements 3 weeks later were considered evaluable for response.

Response, when observed, occurred promptly with a median time from initiation of therapy to response of 7.5 weeks or two and one half drug courses (table 4). This suggests that the greater number of drug courses received by responders was a result of rather than a cause for response. PFI or response duration as well as survival (table 5) were significantly different among the various response categories. These comparisons are summarized in table 6 and basically indicate that the more complete the response, the better the response duration and survival.

Prognostic Factors

A number of different factors of possible prognostic importance were examined since this study is the largest single trial of cytotoxic drugs in endometrial carcinoma. There were no discernible differences among the response categories with regard to age and time from initial diagnosis to first recurrence (table 7). PS at the time of entry in the study did have an impact on likelihood of response in that 15 of 33 patients with an initial PS of 0 or 1 had a response compared to only one of ten patients with a PS of 2 or 3; the difference was significant ($P < 0.05$) (table 8). Histologic grade as determined by a review of pathology slides by a panel of pathologists did not correlate with response to therapy with adriamycin (table 9). Tissue from areas of metastases was not available in every case; in these cases, grade was determined by a review of the pathology of the primary lesion. Similarly, the site of metastatic disease did not appear to influence the likelihood of response (table 10). Previous therapy with surgery, radiation, or progestational agents also exerted no influence on the likelihood of response (table 11).

TABLE 4.—Duration of response*

	Time to response (wks)		No. of courses to response		No. of courses received	
Response	Median	Range	Median	Range	Median	Range
CR	9	3–15	3	1–5	8	6–10
PR	6	3–15	2	1–5	7	5–9
SD	—	—	—	—	5	2–10
PD	—	—	—	—	2.5	1–5
Totals	7.5	3–15	2.5	1–5	3	1–10

*Time to response was generally prompt. The median No. of drug courses to response is equal to the median No. of courses received by those patients with PD, an observation which suggests that patients were given a reasonable trial of adriamycin.

TABLE 5.—Duration of response and survival*

	Duration of response (mos)		Survival (mos)	
Response	Median	Range	Median	Range
CR	7.4	4–10	14.0	6–15
PR	4.4	3–8	6.8	3+–11+
SD	3.8†	1–12+†	6.3	1–12+
PD	3.0†	1–6†	3.5	1–6+
Totals	—	1–12+	6.8	1–15

*Duration of response or, where appropriate, PFI and survival are given for each response category. Contrary to most data concerning the use of single cytotoxic agents, survival advantage is present for responders to adriamycin in patients with endometrial carcinoma (*see* text).

†Refers to PFI rather than response duration.

TABLE 6.—Comparisons of response duration and survival showing significant differences*

CR vs PR + SD + PD
CR + PR vs SD + PD
CR + PR + SD vs PD†
CR vs PR vs SD vs PD

*Comparisons of response duration (or PFI) and survival both demonstrated statistically significant differences in the 4 groupings above. In each case, those patients with CR had results significantly superior to results in patients in other categories. (All differences at $P = 0.001$ level except survival for 3rd comparison [†] which was at $P = 0.01$ level.)

TABLE 7.—Relationship of other factors to response*

	Age (yrs)		Time to 1st recurrence (mos)	
Response	Median	Range	Median	Range
CR	62	45–74	15.5	1–175
PR	65	57–73	17	3–46
SD	68	51–81	19	9–101
PD	64	54–75	18	2–67
Totals	64	45–81	18	1–175

*Age and time from initial diagnosis to 1st recurrence appear to have no effect on likelihood of response.

TABLE 8.—Relationship of PS to response*

Response	No. of patients with PS—				Totals
	0	1	2	3	
CR	2	9	0	0	11
PR	2	2	1	0	5
SD	4	4	4	1	13
PD	1	9	4	0	14
Totals	9	24	9	1	43

*It should be noted that 15 of 33 patients with an initial PS of 0 or 1 responded with a CR or PR while only 1 of 10 patients with an initial PS of 2 or 3 so responded. This difference is significant at $P < 0.05$.

TABLE 9.—Relationship of histologic grade to response*

Response	No. of patients with histologic grade—				Totals
	0	1	2	3	
CR	0	8	3	0	11
PR	0	2	2	1	5
SD	2	3	7	1	13
PD	2	5	6	1	14
Totals	4	18	18	3	43

*Histologic grade, determined from review by a panel of pathologists of tissue from the metastatic site, when available, or from the primary tumor, did not influence likelihood of response.

TABLE 10.—Relationship of metastasis to response*

Response	No. of patients with site of metastasis in—			Totals
	Pelvis	Outside pelvis	Both	
CR	5	4	2	11
PR	4	0	1	5
SD	5	4	4	13
PD	5	4	5	14
Totals	19	12	12	43

*The location of disease, whether confined to the pelvis or in more distant sites, had no influence on likelihood of response. Nos. of patients with disease at any particular metastatic focus were too small to permit any conclusions about the influence of specific metastatic site on response. Patients with pelvic disease were evaluated primarily by pelvic examination.

TABLE 11.—Relationship of previous therapy to response*

	Previous therapy					
	Surgery		Radiation		Progestins	
Response	No. of patients	%	No. of patients	%	No. of patients	%
CR	7	28	10	28.6	3	18.8
PR	3	12	5	14.3	5	31.2
SD	7	28	8	22.9	2	12.5
PD	8	32	12	34.2	6	37.5
Totals	25	100	35	100	16	100

*Previous therapy, whether with surgery, radiation, or progestational agents, did not influence response rates.

Toxic Effects

Hematologic toxicity was the most commonly reported adverse effect with 21 of 43 patients noted to have some evidence of myelosuppression. Of 21 patients with leukopenia (table 12), the point of lowest recorded leukocyte count was noted in 12 patients, with the median day of occurrence on Day 15 following administration of drug (range, Days 4–28). The median drug course in which the lowest leukocyte count was recorded was course 2 (range, 1–6). Of five patients with thrombocytopenia (table 13), three had the point of lowest recorded platelet count noted (Days 4, 8, and 15); these occurred in courses 1, 3, and 5 respectively.

Cardiac toxicity was noted in five of 43 patients. In two of these patients premature atrial contractions represented the only manifestation of possible cardiac toxicity. No other arrhythmias were noted. CHF was seen in three of the 43 patients. In the first of the three patients, CHF was noted prior to the first dose of adriamycin; in the second, it was noted after a cumulative dose of 360 mg/m^2; and in the third, it was noted after a cumulative dose of 540 mg/m^2. The only cardiac death occurred in this third patient, who was the only patient in the study to receive more than the specified maximum cumulative dose of 500 mg/m^2.

Other toxic effects that were noted included: nausea and vomiting of a moderate degree (ten patients), alopecia totalis (nine patients), local skin necrosis because of extravasation (two patients), and stomatitis, weakness, and pneumonia (one case each). No deaths were noted as a result of adverse effects except for the single cardiac death.

DISCUSSION

A recent review of the literature (4) reveals that no concerted effort has been taken to study the activity of cytotoxic drugs in advanced or recurrent endometrial carcinoma. Data collected from broad phase II studies of single agents reveal three agents which might have activity in the treatment of the disease: cyclophosphamide, 5-fluorouracil, and adriamycin (table 14). From this group, adriamycin was selected for extensive phase II testing by the GOG because its suggested level of activity appeared to be greater than that of the other agents.

Adriamycin is a 14-hydroxyl derivative of daunorubicin, an antitumor antibiotic isolated from cultures of *Streptomyces peucetius*. It has been shown to have activity against a wide variety of tumors, but it also causes potentially fatal cardiotoxicity which is dose-related and increases in frequency at cumulative doses of > 550 mg/m^2 (5,6). As noted above, its activity in advanced or recurrent endometrial carcinoma has been suggested in a small number of patients collected from three studies (4).

The results of the current trial clearly document

TABLE 12.—Relationship of leukopenia to response*

	No. of patients with leukopenia grade—					
Response	0	1	2	3	4	Totals
CR	2	0	4	5	0	11
PR	2	0	3	0	0	5
SD	10	2	0	1	0	13
PD	8	1	3	0	2	14
Totals	22	3	10	6	2	43

**See* table 2 for definition of grade. Leukopenia was noted in almost half of the patients.

TABLE 13.—Relationship of thrombocytopenia to response*

Response	No. of patients with thrombocytopenia grade—					Totals
	0	1	2	3	4	
CR	10	0	1	0	0	11
PR	5	0	0	0	0	5
SD	12	0	1	0	0	13
PD	11	2	0	0	1	14
Totals	38	2	2	0	1	43

**See* table 2 for definition of grade. Only 5 patients were noted to have thrombocytopenia.

TABLE 14.—Drugs with possible activity as shown in broad phase II single-agent studies*

Agent	No. of responses/ No. of patients treated	No. of studies reviewed
Cyclophosphamide	7/33 (21%)	5
5-Fluorouracil	10/43 (23%)	7
Adriamycin	7/18 (38%)	3

* From the collected series, the data suggest activity for 3 agents (4).

the activity of adriamycin in the treatment of endometrial carcinoma. The overall objective response rate is 37% (16 of 43 patients), but the CR rate of 26% (11 of 43 patients) is even more striking (table 3). Response occurred reasonably promptly in most cases and was noted by the completion of three cycles of therapy (table 4). Most notably, response resulted in an improved survival, particularly in those patients who achieved a CR (tables 5 and 6).

Since this was the first extensive study of the use of cytotoxic drugs in endometrial carcinoma, a number of factors of possible prognostic importance were examined. Age, time from initial diagnosis to first recurrence, histologic grade, site of metastasis, and previous therapy all appear to have no influence on the likelihood of response. Data on histologic grade did not, however, include evaluation of tissue from metastases in every case so that this factor should be closely evaluated in future studies. Similarly, the influence of specific metastatic sites on prognosis could not be evaluated because of the small numbers of cases in each sub-category. With regard to prior progestational therapy (table 11), a favorable influence of such therapy on subsequent response to cytotoxic therapy is suggested but not demonstrated statistically (eight responses among 16 patients with prior progestins versus eight responses among 27 patients without prior progestins). This point is worthy of attention in future trials which might consider combining progestins and cytotoxic agents.

A factor which does appear to have prognostic significance is patient PS at the time of initiation of cytotoxic therapy (tables 1 and 8). Those patients who had an excellent initial PS of 0 or 1 demonstrated a significantly higher frequency of response (15 responses among 33 patients) than those who had a poor initial PS (one response among ten patients). This observation is similar to observations in patients with a variety of other solid tumors.

Toxicity in this study is similar to that observed in other phase II trials of adriamycin and is acceptable. The most common toxic effect was myelosuppression which mainly affected leukocytes.

In conclusion, adriamycin is now established as an active agent for the treatment of advanced or recurrent endometrial carcinoma with acceptable toxicity. Further trials in combination with other active agents are indicated.

REFERENCES

1. REIFENSTEIN EC. Hydroxyprogesterone caproate therapy in advanced endometrial cancer. Cancer 27:485–502, 1971.
2. KISTNER RW. The use of progestins. *In* Obstetrics and Gynecology. Chicago, Year Book Medical Publishers, 1969, p 129.
3. SMITH JP. Hormone therapy for adenocarcinoma of the endometrium. *In* Cancer of the Uterus and Ovary. Chicago, Year Book Medical Publishers, 1969, pp 73–83.
4. DEVITA VT, JR, WASSERMAN TH, YOUNG RC, ET AL. Perspectives on research in gynecologic oncology: treatment protocols. Cancer 38:509–525, 1976.
5. BLUM RH. An overview of studies in adriamycin (NSC-123127) in the United States. Cancer Chemother Rep Part 3, vol 6(2):247–251, 1975.
6. BLUM RH, and CARTER SK. Review of adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–257, 1974.

cis-Dichlorodiammineplatinum(II), Adriamycin, Cyclophosphamide, CCNU, and Vincristine in Non-Small Cell Lung Carcinoma: A Preliminary Report [1]

Hiroshi Takita,* Patrick C. Marabella, Francis Edgerton, and Donna Rizzo [2]

Synergistic antitumor effects of *cis*-dichlorodiammineplatinum(II)(DDP)with cyclophosphamide(CYC) and other agents have been reported (1,2). In a search for better therapeutic effects, > 100 patients with inoperable non-small cell lung carcinoma were treated with various DDP-based polychemotherapy regimens (3,4). A five-drug combination, DDP, adriamycin (ADM), CYC, CCNU, and vincristine (VCT), was recently found to produce an unusually high response rate in non-small cell lung carcinoma, and the preliminary results in 35 patients are presented.

MATERIALS AND METHODS

Thirty-five patients with inoperable non-small cell lung carcinomas with measurable lesions were entered in the study. There were 15 squamous cell carcinomas, 11 adenocarcinomas (three alveolar cell type), and nine large cell carcinomas (one anaplastic type). The ages of the patients ranged from 31 to 74 years with a median of 58 years, and all but four of the patients were males. Nineteen patients had disease limited to the hemithorax and 16 had disseminated disease. The performance status (Zubrod) was 1 in 19 patients, 2 in 13, and 3 in three. Informed consent was obtained from all patients.

Therapy consisted of 50 mg/m^2 of DDP, 50 mg/m^2 of ADM, 300 mg/m^2 of CYC, 50 mg/m^2 of CCNU, and 1.4 mg/m^2 of VCT given simultaneously and repeated every 4–6 weeks. DDP was dissolved in 1000 ml of 5% dextrose in water containing 37.5 g of mannitol and was given iv in a 4-hour period. An additional 1000 ml of 5% dextrose in water was given iv in 4 hours following infusion of DDP for the purpose of hydration and diuresis. CCNU was given orally and ADM, CYC, and VCT were injected through iv tubing.

Four patients were found to have brain metastasis, and three received radiation therapy of 3000 rads in ten fractions. Two patients with apparent solitary metastasis to the thoracic vertebra received very localized radiation therapy of 3000 rads in ten fractions. One other patient received prior radiation to the primary tumor, also with 3000 rads in ten fractions.

Complete response (CR) is defined as the disappearance of all clinical evidence of tumors. Partial response (PR) is defined as a reduction of > 50% in the sum of the products of the greatest tumor diameter and its perpendicular measurement for all measurable lesions for ≥ 2 months. Stable disease is defined as no progression or a < 50% reduction for ≥ 2 months, and was not included as an objective response. Survival was calculated (in weeks) from the onset of the chemotherapy.

RESULTS

Two to seven courses (mean, 3.1 courses) of therapy have been given. The overall response rate was 65.7% (23 of 35 patients): there were four CRs, 19 PRs, and three patients with stable disease (table 1). In all responders, reduction in the size of the lesion was noted in 4 weeks and the maximum response was noted in 8–12 weeks.

It is of interest that 13 of 15 patients with squamous cell carcinoma responded. Six of eight patients with adenocarcinoma responded, but only three of nine patients with large cell carcinoma showed > 50% tumor regression. Thus, significantly fewer large cell carcinoma patients responded than did patients with other cell types ($P < 0.025$, Fisher's exact test). There was a response in 13 of the 19 patients with limited disease and in ten of the 16 with disseminated disease (table 2). This is an insignificant difference in response rates ($\chi = 0.05$, chi-square test). Performance status and extent of disease (except for brain metastasis) did not seem to affect the response rate.

All of the patients had nausea and vomiting lasting overnight and 15 had diarrhea as acute side effects. Leukopenia of < 2000 cells/mm^3 occurred in five patients, thrombocytopenia of < 100,000 cells/mm^3 in five, and hemoglobin level of < 10 g in seven; the peak of the bone marrow toxicity was

[1]Received Apr 20, 1978; revised Nov 17, 1978; accepted Nov 27, 1978.

[2]Department of Thoracic Surgery, New York State Department of Health, Roswell Park Memorial Institute, Buffalo.

**Reprint requests to:* Hiroshi Takita, MD, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

reached in 2 weeks but these bone marrow toxic effects were transient and reversible. Alopecia of various degrees was observed in all patients. Ototoxicity was evaluated by audiogram every 2 months and there was mild to severe loss in the high-frequency range in only five patients. Renal toxicity was followed by measuring the serum creatinine level; in 12 of 32 patients the level was > 1.2

TABLE 1.—DDP, ADM, CYC, CCNU, and VCT in non-small cell carcinoma of the lung

Cell type	No. of patients with—				
	CR	PR	Stable disease	No response	Response*
Squamous cell carcinoma	2	11		2	13/15
Adenocarcinoma	1	5	1	1	6/8
Large cell carcinoma		3	1	5	3/9
Alveolar cell carcinoma	1		1	1	1/3
Totals	4	19	3	9	23/35

*Total No. of patients responding/No. evaluated.

TABLE 2.—Response to DDP, ADM, CYC, CCNU, and VCT chemotherapy by cell type and extent of disease

Cell type	Extent of disease*	Survival (wks)	Response by disease site†						Total response
			Primary	Lymph nodes	Bone	Lung	Brain	Liver	
Squamous	D	45.4	NR	CR	CR				PR
Squamous	L (+)	13.1	PR	PR					PR
Squamous	L (+)	47.7	CR	CR					CR
Squamous	D	36.7	PR		CR				PR
Squamous	L (+)	8.7	PR						PR
Squamous	D	28.0				PR			PR
Squamous	L	10.9	PR	PR					PR
Squamous	L	32.4	PR						PR
Squamous	L	15.1	NR						NR
Squamous	L	49.9	CR						CR
Squamous	D	32.4	PR			CR			PR
Squamous	L	15.1	SD						SD
Squamous	L	15.1	PR	PR					PR
Squamous	L	23.6	PR						PR
Squamous	L	15.1	PR						PR
Alveolar	D	45.4	CR		CR				CR
Adenocarcinoma	L	23.6	PR	PR					PR
Adenocarcinoma	D	36.7	PR			PR	PR		PR
Adenocarcinoma	D (+)	4.3	CR			CR	NR		PR
Adenocarcinoma	D (+)	17.4	NR	NR	NR				NR
Alveolar	D (+)	21.9	NR			NR			NR
Adenocarcinoma	L	41.4	CR						CR
Adenocarcinoma	D (+)	30.3	PR	PR				PR	PR
Adenocarcinoma	L (+)	45.4	PR	PR					PR
Adenocarcinoma	D (+)	8.7	NR		NR				NR
Alveolar	D (+)	8.7	NR	NR	NR				NR
Large	L	41.1	NR						NR
Large	D (+)	4.4	NR	NR	NR				NR
Large	L	53.0	PR						PR
Large	L (+)	13.1	NR	NR					NR
Large	D (+)	4.4	PR	PR		PR	PR		PR
Large	L	41.1	NR						NR
Large	L (+)	13.0	NR						NR
Large	D (+)	8.7	NR	NR	NR				NR
Large	D	45.4	CR	PR			NR		PR

*L = limited to hemithorax; D = disseminated; (+) = death.
†SD = stable disease; NR = no response.

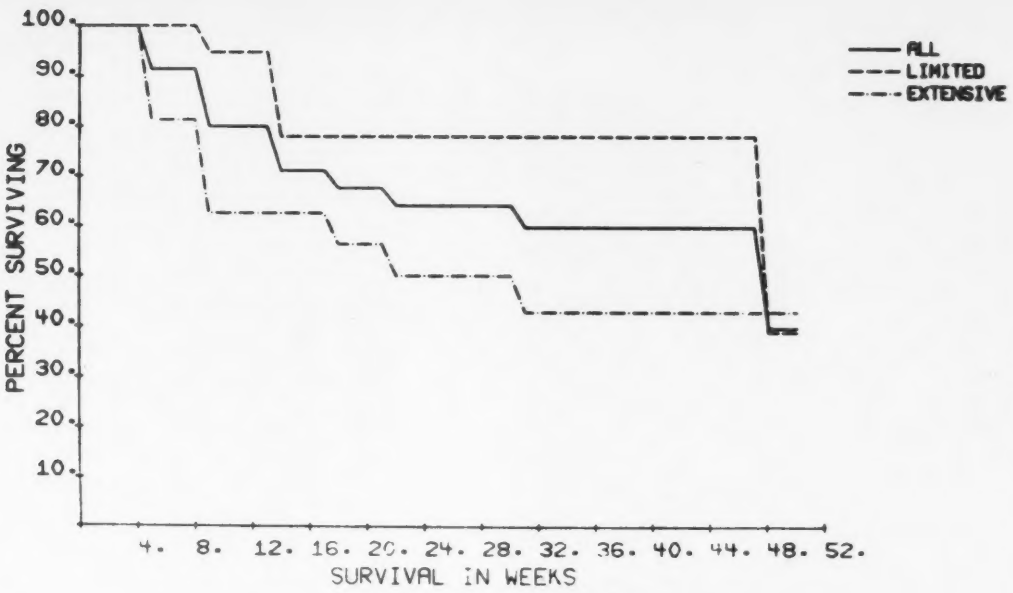


FIGURE 1.—Survival by extent of disease.

mg/100 ml, with none > 2.0 mg/100 ml. For administration of chemotherapy, the patients were usually admitted overnight to the hospital because of gastrointestinal side effects (nausea and vomiting). Five patients required hospitalization: two for treatment of pulmonary infection and three for general supportive care. One patient died at home 4 days after the first dose of chemotherapy, apparently a sudden death (cause unknown); this case is not included in the report.

Actuarial life-table analysis and the Breslow test of failure rates were used to evaluate survival differences among various subgroups. This statistic is concerned with the difference between two or more distributions of failure over time. The median survival times may be derived directly from figures 1–3 by construction of a 50% survival line. The median is determined by the actuarial method when one half of the patients observed across a given time period are known to have failed prior to or during that period. That other patients known to be alive have not been observed for that length of time results in this figure being an estimate since continued observance of these patients may alter the distribution of known failures (5).

In the current study, the longest followup has been 53 weeks with an estimated median survival for all patients of 47.5 weeks. Table 3 summarizes the estimated median survival times for various subgroups. The results of the Breslow tests were as follows: patients with limited disease had a significantly better survival distribution ($P < 0.05$) than patients with disseminated disease, squamous cell patients had a significantly better distribution than those with large cell carcinoma ($P < 0.05$), and patients who responded to therapy had a significantly better distribution than nonresponders ($P < 0.01$). At the time of analysis, 14 patients had died of progressing disease.

DISCUSSION

Numerous chemotherapeutic agents have been known to have limited effectiveness against non-small cell lung carcinoma, but it is possible that we

TABLE 3.—Subgroup median survival times

Group	No. of patients	Median survival in wks
Total	35	47.5 (estimated)
Limited disease	19	71.4 (projected)*
Disseminated disease	16	29.5 (estimated)
Squamous cell	15	47.8 (estimated)
Adenocarcinoma	11	30.4 (estimated)
Large cell	9	13.8 (estimated)
Responders	23	79.0 (projected)*
Nonresponders	12	16.5 (estimated)

* Figures are based on an exponential model and should be regarded as especially unreliable.

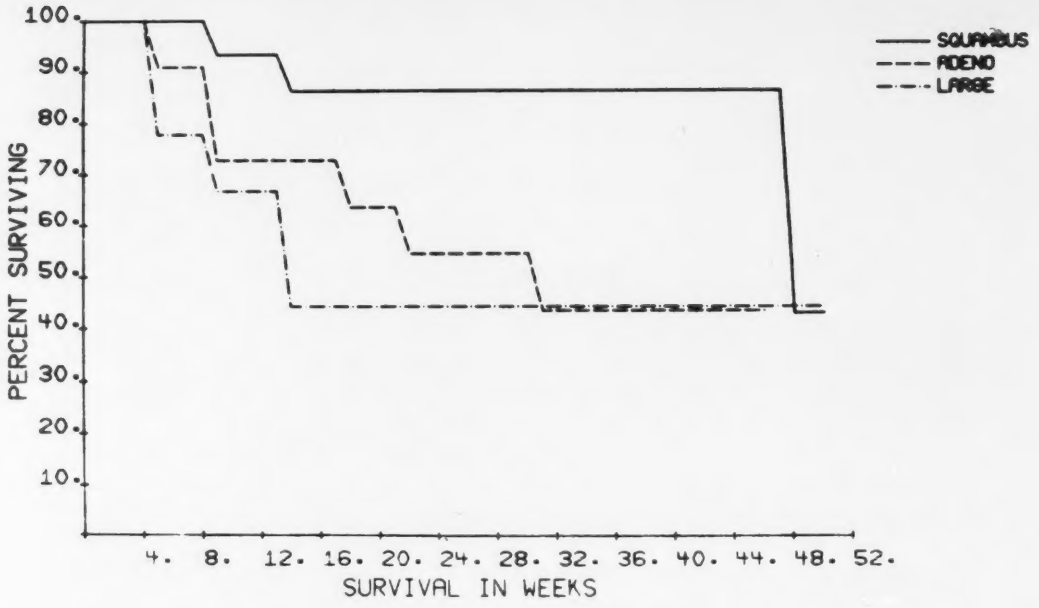


FIGURE 2.—Survival by cell type.

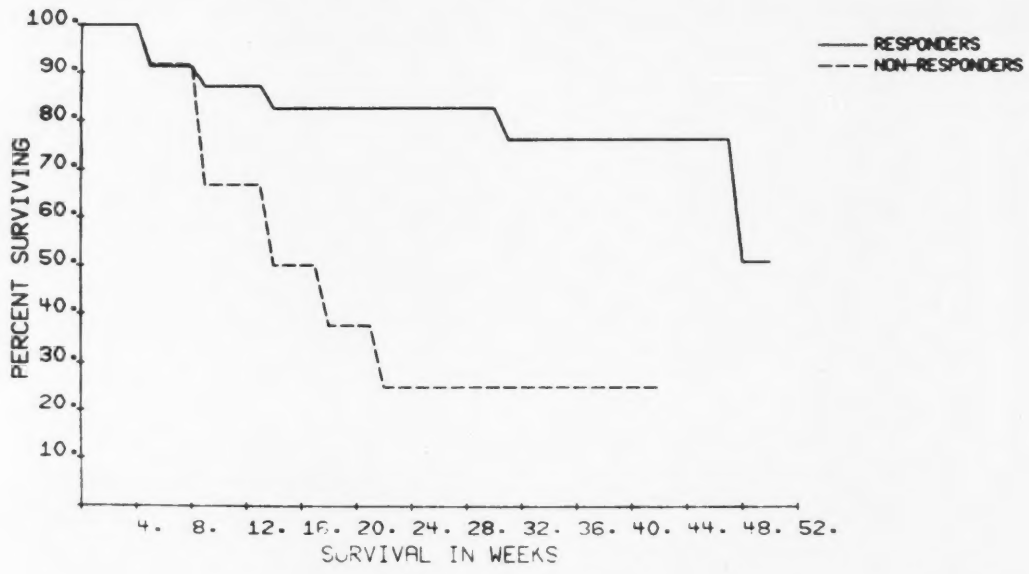


FIGURE 3.—Survival by response to therapy.

still may not know how to use them in order to obtain the best possible therapeutic effects (6). Our previous clinical study with DDP, ADM, CYC, and VCT in non-small cell lung carcinoma showed a response rate of 18% with a median survival of 26 weeks, but, at times, we observed a striking reduction in tumor size (3). The addition of CCNU to the above four drugs increased the response rate by two to three times. Therefore, the possibility arises that CCNU has a synergistic effect in combination

with DDP, ADM, CYC, and VCT, or in some combination of these agents.

In this reported series, an administration schedule of once every 4–6 weeks was chosen pragmatically (most of the patients were from distant areas), but fortunately it appears to have been a rather good schedule for non-small cell lung carcinomas which are rather slow growing. Several patients with small cell lung carcinoma were treated with the same regimen, but we found this to be unsuitable because of the faster growth rate of small cell carcinoma. There was an initial regression of the tumor, followed by progression 3–4 weeks after chemotherapy. This may indicate the need for a more frequent administration of chemotherapy in small cell carcinoma.

Because of these preliminary encouraging results in non-small cell lung carcinoma, further clinical study continues.

REFERENCES

1. PAGE RH, TALLEY RW, and BUHAGIAR J. The enhanced antitumor activity of *cis*-diamminedichloro platinum (II) against murine tumors when combined with other agents. J Clin Hematol Oncol 7:96–104, 1977.
2. PAGE RH, TALLEY RW, and LIVERMORE DH. The effect of *cis*-diamminedichloro platinum (II) and cyclophosphamide on immune response and tumor rejection in BALB/c and PL/Jax mice. J Clin Hematol Oncol 7:105–113, 1977.
3. BJORNSSON S, TAKITA H, KUBERKA N, ET AL. Combination chemotherapy plus methanol extracted residue of bacillus Calmette-Guérin or *Corynebacterium parvum* in stage III lung cancer. Cancer Treat Rep 62:505–510, 1978.
4. TAKITA H, HOLLINSHEAD AC, and BJORNSSON S. Chemotherapy, surgery and immunotherapy of inoperable lung cancer. Proc Am Assoc Cancer Res and ASCO 19:319, 1978.
5. PETO R, PIKE MC, ARMITAGE P, ET AL. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1–39, 1977.
6. SELAWRY OS. The role of chemotherapy in the treatment of lung cancer. Semin Oncol 1:259–272, 1974.

Animal Models for the Comparative Assessment of Neurotoxicity Following Repeated Administration of Vinca Alkaloids [1]

Glen C. Todd,* William J. Griffing, William R. Gibson, and Douglas M. Morton [2]

SUMMARY

Neurotoxicity is the major side effect occurring during the clinical use of vincristine (VCR). Animal models predictive of potential neurotoxicity would be very useful in the preclinical development of new vinca compounds. To simulate conditions in which neurotoxicity is produced during the clinical use of VCR, experimental animals (except the guinea pig) were given the test compounds by the iv route over a prolonged time period. Doses were selected based on the production of leukopenia. Vindesine (VDS), a chemically modified vinblastine (VBL) product, was compared with VCR and VBL in animal studies. Definite neurotoxic manifestations developed when VCR was given to chickens, cats, and monkeys. The administration of VDS or VBL did not produce neurotoxic signs in these species. The mouse, rat, dog, and guinea pig were not found to be useful models. Thus, it would appear the chicken, cat, and monkey would be appropriate animal models for the preclinical testing of new vinca compounds.

[Cancer Treat Rep 63:35–41, 1979]

A new chemically modified vinca alkaloid, vindesine (VDS), was studied in mice, rats, and dogs (1). The primary toxic effects were: anorexia, diarrhea, leukopenia, erythropenia, elevated lactic dehydrogenase, and skeletal muscle degeneration. In addition to defining the general signs of toxicity caused by vinca compounds, we have attempted to find an animal model which would develop neurotoxicity resembling that produced in man during the clinical use of vincristine (VCR) (2–4). The prerequisites of such a model would be the development of neurotoxicity while receiving iv sublethal leukopenic doses over an extended period of time. With such an animal model a direct comparison could be made between VCR and new vinca compounds.

In previous studies, mice, rats, and dogs did not develop neurotoxic effects when given VCR (1). In this paper we report the comparative findings in the chicken, guinea pig, cat, and monkey. Species which developed neurotoxic effects when given repeated sublethal doses of VCR were the chicken, cat, and monkey. The chicken was especially useful because the signs of toxicity were distinct and different for VCR, vinblastine (VBL), and VDS. When given doses of VCR for 5–7 months, the monkey developed clinical signs of neurotoxicity which resembled those found in man. Guinea pigs did not develop leukopenia or neurotoxic effects.

MATERIALS AND METHODS

All toxicity studies were performed using VDS, VCR, or VBL as their sulfate salts diluted with sterile saline.

Chicken

Young White Rock chickens weighing 1.5–2.0 kg were separated into four treatment groups consisting of four males and four females. The test compounds were administered iv twice a week for 1 month according to the schedule described in table 1. Doses of 0.8, 1.6, or 2.4 mg/kg are equivalent to 8.8, 17.6, or 26.4 mg/m^2, respectively.

The chickens were examined daily for changes in

[1]Received May 2, 1978; revised Oct 16, 1978; accepted Nov 28, 1978.

[2]Department of Pathology, Lilly Research Laboratories, Division of Eli Lilly and Co, Greenfield, Ind.

Reprint requests to: Glen C. Todd, PhD, Lilly Research Laboratories, Division of Eli Lilly and Co, PO Box 708, Greenfield, Ind 46140.

TABLE 1.—Drug dose and schedule in chickens

Wk	mg/kg/injection			
	Diluent control	VDS	VCR	VBL
1	0.8	0.8	0.4	0.8
2	1.6	1.6	0.4	1.6
3	2.4	2.4	0.4	1.6
4	2.4	2.4	0.8	1.6
Total cumulative dose (mg/kg)		14.4	4.0	11.2

physical condition, appetite, and behavior, and for signs of altered locomotor ability. Chickens that became severely depressed or developed reduced locomotor activity were killed for necropsy and the tissues were examined by light and electron microscopy.

Guinea Pig

Dose-ranging studies were performed in order to identify repeated sublethal doses of VDS, VCR, or VBL which would produce leukopenia in guinea pigs. Such doses were not achieved because intestinal toxicity and death occurred prior to a leukopenic response.

Guinea pigs weighing 326–428 g were separated into four treatment groups consisting of eight males and eight females and were given the test compounds sc twice a week for 12 weeks. The doses (mg/kg/injection) by group were: diluent control; VDS, 0.07; VCR, 0.05; and VBL, 0.07. The VDS and VBL doses were reduced to 0.05 mg/kg/injection after 2 weeks because of toxicity and excessive body weight loss. The cumulative dose (mg/kg) given per animal in each group was: VDS, 1.28; VCR, 1.2; and VBL, 1.28 (a dose of 0.05 mg/kg is equivalent to 0.35 mg/m^2). When an animal stopped eating, the subsequent dose was not administered. The animals were bled prior to the test period and five times during the study for determination of leukocyte and erythrocyte counts. Two males and two females from each group were necropsied and the tissues were examined microscopically.

Cat

Studies were performed in cats given iv doses of vinca compounds which would produce leukopenia (≥ 50% reduction in leukocytes) with a minimal mortality rate. Adult domestic cats weighing 2.0–4.9 kg were separated into four groups and were given doses twice weekly according to the design shown in table 2. Doses of 0.03 or 0.06 mg/kg are equivalent to 0.33 or 0.66 mg/m^2, respectively. The cumulative dose (mg/kg) per group in cats treated for 12 weeks was: VDS, 1.44; VCR, 0.72; and VBL, 1.44.

TABLE 2.—Drug and dose schedule in cats

Treatment	No. of cats/group		Dose (mg/kg/injection)
	Males	Females	
Diluent control	4	4	0.2 (ml/kg)
VDS	5	5	0.06
VCR	7	7	0.03
VBL	5	5	0.06

The test animals were observed for changes in behavior, physical condition, and appetite, and for neuromuscular activity or capability. Hematologic and blood chemistry values were determined prior to the start of the study and at five intervals during the test period. Cats from each group were necropsied after 8 or 12 weeks on the test. Treatment was discontinued in selected cats after 12 weeks and they were allowed a 4-week recovery period before necropsy. Tissues were prepared for light and electron microscopic evaluation.

Monkey

Four acclimated young adult male rhesus monkeys weighing 4.7–6.7 kg were separated into two groups. Two animals received VDS and two received VCR for 9 months. Iv doses were given once a week according to the schedule shown in table 3. Doses of 0.25 or 0.3 mg/kg are equivalent to 3.0 or 3.6 mg/m^2, respectively.

The monkeys were observed daily for changes in behavior, physical appearance, and appetite, and for neuromuscular activity. Because severe signs of neurotoxicity developed in one monkey given VCR, treatment was discontinued during the sixth and seventh test months. Hematologic and blood chemistry values were determined prior to the test and at periodic intervals during the study. The monkeys were necropsied and tissues were prepared for evaluation by light and electron microscopy.

TABLE 3.—Drug and dose schedule in monkeys

Duration of treatment (wks)	mg/kg/injection	
	VDS	VCR
1–7	0.1	0.1
8–15	0.2	0.2
16–25	0.25	0.25
26–38	0.3	0.25
Total cumulative dose (mg/kg)	8.7	8.05

TABLE 4.—Comparative findings in chickens treated with VDS, VBL, or VCR

Observation	Test groups (incidence/8 chickens)			
	Control	VDS	VBL	VCR
Hypoactivity	0	3	1	6
Anorexia	0	2	1	5
Reduced body weight gain	0	7	8	8
Progressive pallor	0	6	8	4
Lethargy	0	6	0	0
Extreme pallor	0	0	8	0
Marked ataxia	0	0	0	7
Skeletal muscle degeneration and necrosis	0	2	1	4
Death	0	3	7	7

RESULTS

Chicken

Table 4 gives the comparative results in chickens treated with VDS, VBL, or VCR. Common to all groups receiving the vinca compounds were periods of hypoactivity, anorexia, reduction in body weight gain, and progressive development of pallor. The distinguishing characteristics of the three test compounds in chickens were: VDS, lethargy; VBL, extreme pallor; and VCR, neurotoxic effects. Chickens receiving VCR developed severe signs of neuromuscular toxicity consisting of ataxia, wing-drop, and an inability to stand, walk, or hold head erect. Deaths occurred in all test groups between Days 11 and 27 of the test. Lesions in nerve tissues were not found by light or electron microscopy in chickens. However, a low incidence of skeletal muscle degeneration and necrosis was present.

Guinea Pig

All groups receiving the test compounds developed hypoactivity, anorexia, marked body weight loss, and death, without physical signs of reduced neuromuscular activity. The mortality incidence in guinea pigs was: one of 16 controls, six of 16 treated with VDS, four of 16 treated with VBL, and four of 16 treated with VCR. Deaths occurred between Days 6 and 46 of the test. Although the doses were in the lethal range there was no reduction in leukocyte or erythrocyte counts, which is peculiar to the guinea pig. The significant pathologic findings were similar for all groups; they consisted of enlargement of the duodenum, peritonitis, and inhibition of spermatogenesis. The bone marrow was cellular in animals from all test groups. The incidence of focal enlarged axons (for description *see* the cat results) found in the peripheral nerves and spinal cords of the guinea pigs examined were: none of four controls, none of four treated with VDS, two of four treated with VBL, and two of four treated with VCR.

Cat

Table 5 gives the comparative findings in cats treated with VDS, VBL, or VCR. Anorexia, diarrhea, hypoactivity, and weight loss were common physical signs of toxicity. Indicative of neurotoxic effects were definite signs of ataxia. Ataxia was observed in some of the cats in all treated groups but the highest incidence occurred in the VCR group. The vinca compounds at the doses used caused approximately a 50% decrease in the leukocyte and erythrocyte counts. Both blood cell parameters returned to a normal range in the cats allowed a 4-week recovery period. Blood chemistry determinations were not indicative of toxicity. Deaths occurred in all test groups between Days 18 and 82 of the test. The pathologic findings were similar for all vinca test groups, consisting of inhibition of spermatogenesis and a few greatly enlarged axons in the myelinated tracts of the peripheral nerves, spinal cord, and brain stem (table 5). The axonal lesions consisted of segmental enlargements which were homogeneous or fibrillar and argentophilic without significant changes in the myelin sheath or evidence of axonal degeneration distal to the enlargements (fig 1). Electron microscopic examination of nerve tissues revealed a decreased number of microtubules and an increased number of twisted, disorderly neurofilaments (fig 2). A few axonal lesions were still present following the 1-month recovery period.

Monkey

The only physical sign of toxicity which developed in the two monkeys given VDS was an increased propensity for bleeding from the gums following handling and restraint during the last 3 months of the study. Neither monkey developed signs of neu-

TABLE 5.—Comparative findings in cats treated with VDS, VBL, or VCR

Observation	Test groups (No. of observations/No. of cats)			
	Control	VDS	VBL	VCR
Ataxia	0/8	2/10	1/10	6/14
Reduction in leukocytes	0/8	7/10	8/10	12/14
Reduction in erythrocytes	0/8	5/10	8/10	8/14
Inhibition of spermatogenesis	0/4	6/6*	3/3†	6/7
Enlarged axons	0/8	9/9†	6/10	13/13†
Death	0/8	3/10	1/10	4/14

*1 cat listed as female was male.
†Histologic sections were not available for examination from all cats because of autolysis.

rotoxicity during treatment with VDS. One monkey died after 6 months on the test with acute gastric distention (5).

However, both monkeys given VCR had definite physical signs indicative of neurotoxicity. After 5 months of VCR treatment, one monkey evidenced marked incoordination, a reluctance to move, and an inability to use hindlegs. These signs were followed by loss of use of the hands with contraction of the flexor muscles and the formation of a permanent fist (fig 3). The animal lost weight and was removed from treatment for 2 months for a recovery period. The signs of neurotoxicity were partially reversed during this time. When dosing was reinstituted, the signs recurred. The second monkey receiving VCR also developed signs of neurotoxicity, to a lesser degree, after 6½ months in the study.

Monkeys given VDS and VCR had a decrease in myelocytes causing a ≥ 50% reduction in leukocytes. No important alterations occurred in the blood chemistry values. The pathologic findings consisted of inhibition of spermatogenesis and a few greatly enlarged axons in the myelinated areas of the peripheral nerves, spinal cord, and brain stem. The axonal lesions resembled those found in cats.

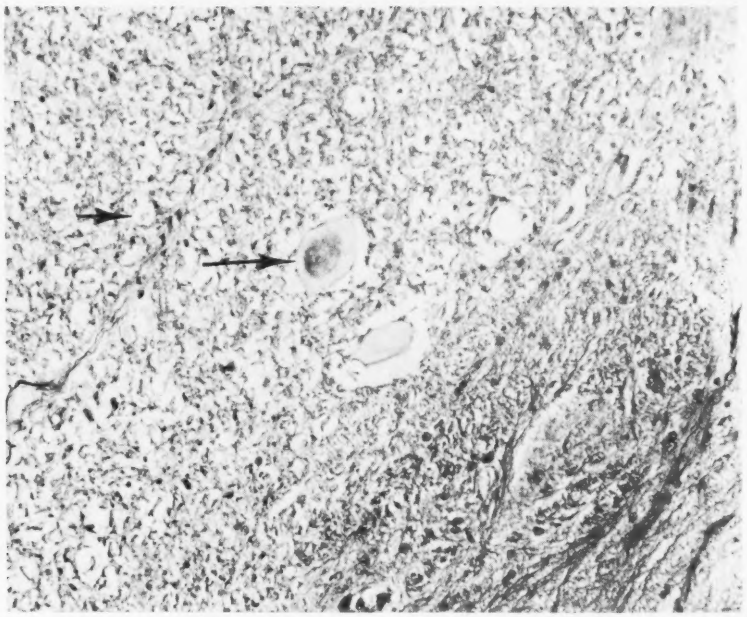


FIGURE 1.—Photomicrograph of the spinal cord from a cat given VCR. There are many cross-sectioned normal myelinated nerve fibers with the axons stained black (small arrow) and 2 greatly enlarged abnormal axons (large arrow). Bielschowsky's silver stain, original magnification × 200.

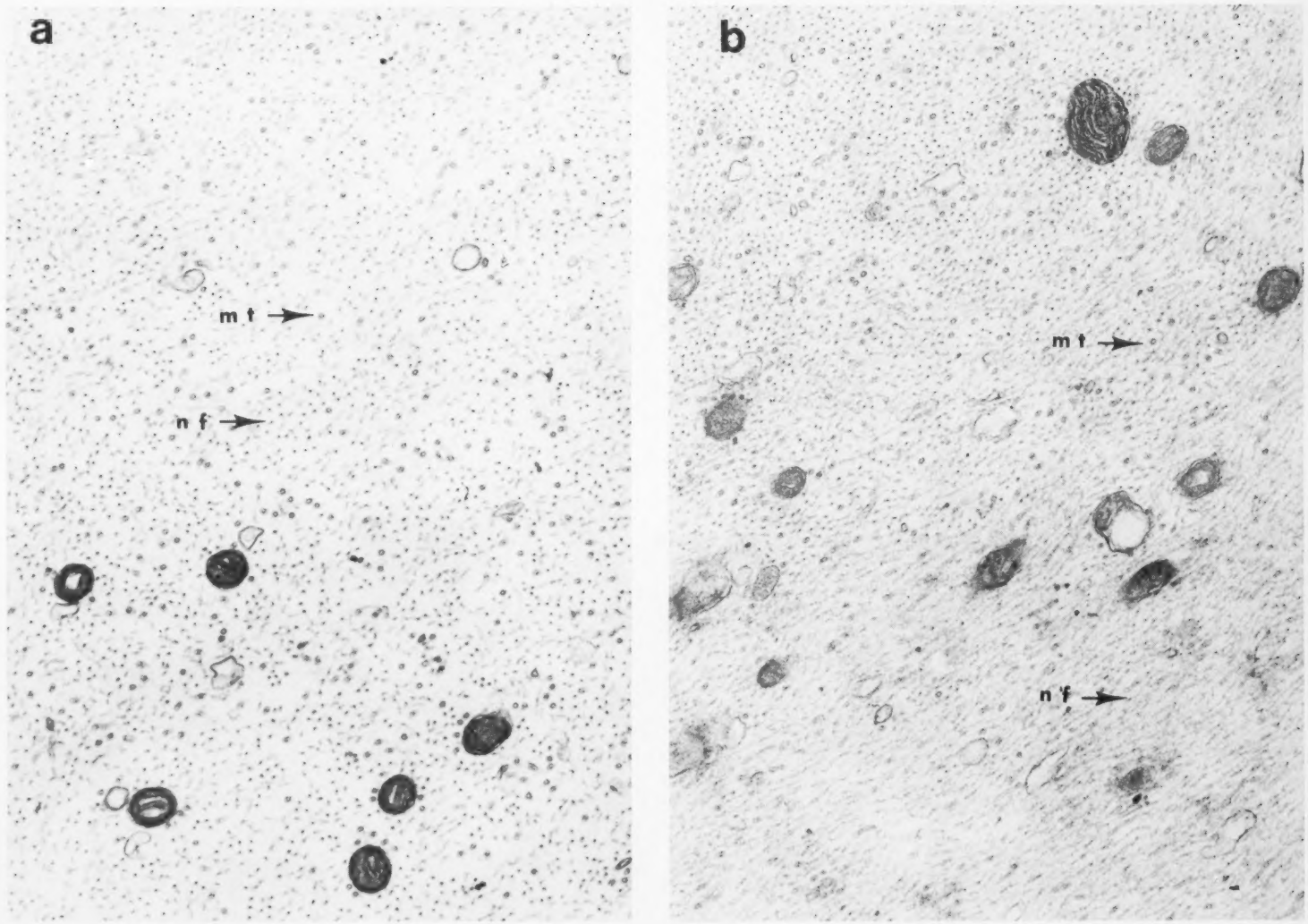


FIGURE 2.—(A) Electron micrograph of the sciatic nerve of a control cat. Note the distribution of the microtubules (mt) and neurofilaments (nf). Uranyl acetate and lead citrate stain, magnification × 43,000. (B) Electron micrograph of the sciatic nerve of a cat treated with VCR. Note the reduction in microtubules (mt) and the increased number of twisted, disorderly neurofilaments (nf). Uranyl acetate and lead citrate stain, magnification × 43,000.

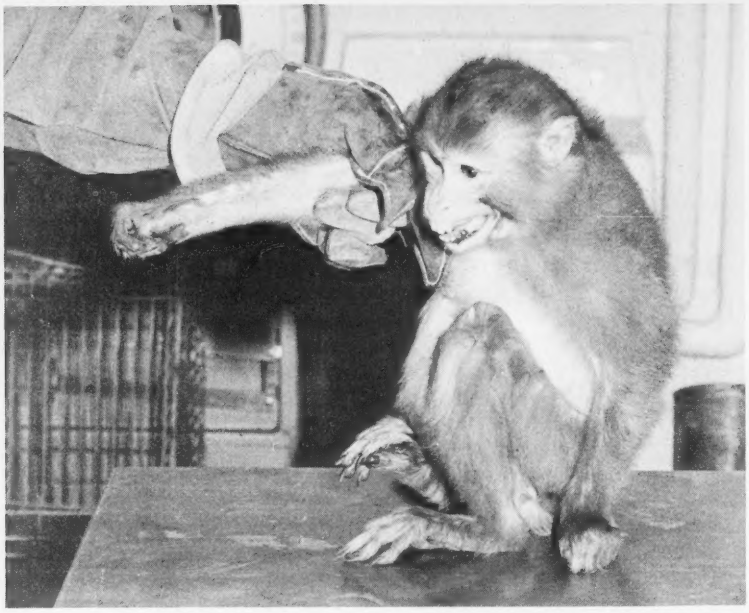


FIGURE 3.—Rhesus monkey given VCR for 6 mos with inability to use hindlegs and hands. Note contraction of flexor muscles and the formation of a fist. These signs were partially reversible during a recovery period of 2 mos.

DISCUSSION

Peripheral neuropathy is recognized as the major limiting factor in the clinical use of VCR (2–4). Minor chemical alteration of the vinca alkaloid structure can significantly change the oncolytic spectrum and toxicity (6–9) which suggests that compounds could be prepared possessing chemotherapeutic potential without significant clinical neurotoxicity. For the preclinical development of new vinca compounds, animal models were needed for the assessment of neurotoxicity. Although other investigators have reported on the neurotoxicity of vinca compounds, most can be faulted for using single lethal doses, placing the test compounds directly into nerve tissues, confusing severe toxic debility with neurotoxicity, failure to compare the vinca compounds under similar test procedures, and failing to correlate clinical signs with morphologic lesions.

In the present studies, the chicken proved to be a very useful animal model. This species developed easily recognized physical signs of neurotoxicity when given VCR, and these signs were distinct from those seen in chickens given VDS or VBL. All three compounds produced skeletal muscle degeneration as the major morphologic lesion. Skeletal muscle degeneration has been reported in animals receiving vinca compounds (1,10).

Guinea pigs given vinca compounds developed severe signs of toxicity involving the intestinal tract. Of particular interest was the failure to produce leukopenia in guinea pigs. They did not develop signs of neurotoxicity although a few had microscopic lesions in nerve tissues resembling those found in cats.

Cats and monkeys developed signs of neurotoxicity when given VCR and had unusual morphologic axonal lesions in the peripheral nerves, spinal cord, and brain stem. A few axons had segmental enlargements, without significant damage to the myelin sheath or lesions in the axons proximal or distal to these enlargements. Similar axonal changes have been reported in rats with chronic vitamin E deficiency (11) and in man with thiamine deficiency (12). Electron microscopy revealed a decreased number of microtubules and an increased number of neurofilaments. This effect on microtubules and neurofilaments has been frequently reported as a major effect of the vinca compounds (13–19). The relationship of uptake and effectiveness to block axoplasmic transport produced in desheathed nerves may account for the greater neurotoxicity of VCR com-

pared to VDS (20–22). Gottschalk et al (23) reported axonal enlargements and degeneration in myelinated nerves in rats and patients given VCR. Focal enlargements in axons of vinca-treated animals are considered specific lesions; this is morphologic evidence of nerve damage. However, a correlation between morphologic alteration in nerves and functional signs of neurotoxicity was less than perfect in these studies.

The rhesus monkey developed physical signs of neurotoxicity when given iv doses of VCR over a long period of time (5–7 months), and the signs of neurotoxicity were reversible during the time VCR was not administered. Folk et al (24) reported the signs of toxicity and described neuronal changes and peripheral neuropathy in monkeys given VCR. The response in monkeys, including leukopenia and signs of peripheral neuropathy and reversibility, resembled that found in man during clinical use of VCR.

Recently, Dyke and Nelson (25) reported on the usefulness of VDS in clinical therapy. They observed paresthesia without ataxia, but this symptom was not progressive as would have been expected with the continued use of VCR. To date, they have not been required to discontinue VDS therapy because of neurotoxicity.

Thus, it is concluded that chickens, cats, and monkeys given iv sublethal leukopenic doses of vinca compounds over a prolonged period of time are useful for the selection of compounds which have less potential for producing clinical neurotoxicity.

REFERENCES

1. TODD GC, GIBSON WR, and MORTON DM. Toxicology of vindesine (desacetyl vinblastine amide) in mice, rats, and dogs. J Toxicol Environ Health 1:843–849, 1976.
2. CASEY EB, JELLIFE AM, LEQUESNE PM, ET AL. Vincristine neuropathy: clinical and electro-physiological observations. Brain 96:69–86, 1973.
3. ROSENTHAL S, and KAUFMAN S. Vincristine neurotoxicity. Ann Intern Med 80:733–737, 1974.
4. WEISS HD, WALKER MD, and WIERNIK PH. Neurotoxicity of commonly used antineoplastic agents. N Engl J Med 291:75–81, 127–133, 1974.
5. NEWTON WM, BEAMER PD, and RHOADES HE. Acute bloat syndrome in stumptail macaques (*Macaca artoides*): a report of four cases. Lab Anim Sci 21:193–196, 1971.
6. SWEENEY MJ, CULLINAN GJ, POORE GA, ET AL. Experimental antitumor activity of vinblastine amides. Proc Am Assoc Cancer Res and ASCO 15:37, 1974.
7. CULLINAN GJ, GERZON K, POORE GA, ET AL. Vinblastine modifications; desacetyl-VLB amides. (Abstr SC-19) *In* Proceedings of the 9th International Congress of Chemotherapy. London, 1975.
8. TODD GC, GIBSON WR, GRIFFING WJ, ET AL. The preclinical study of desacetyl vinblastine amide (DVA). Proc Am Assoc Cancer Res and ASCO 16:70, 1975.
9. BARNETT CJ, CULLINAN GJ, GERZON K, ET AL. Structure activity relationships of dimeric *Catharanthus* alkaloids. I. Desacetyl vinblastine amide (vindesine) sulfate. J Med Chem 21:88–96, 1978.
10. BRADLEY WG. The neuromyopathy of vincristine in the guinea pig: an electrophysiological and pathological study. J Neurol Sci 10:133–162, 1970.
11. PENTSCHEW A, and SCHWARZ K. Systemic axonal dystrophy in vitamin E deficient adult rats. Acta Neuropathol 1:313–334, 1962.
12. TAKAHASHI K, and NAKAMURA H. Axonal degeneration in beriberi neuropathy. Arch Neurol 33:836–841, 1976.
13. SCHOCHET SS, JR, LAMPERT PW, and EARLE KM. Neuronal changes induced by intrathecal vincristine sulphate. J Neuropathol Exp Neurol 27:645–658, 1968.
14. SEIL FJ, and LAMPERT PW. Neurofibrillary tangles induced by vincristine and vinblastine sulfate in central and peripheral neurons in vitro. Exp Neurol 21:219–230, 1968.
15. WISNIEWSKI H, SHELANSKI ML, and TERRY RD. Effects of mitotic spindle inhibitors on neurotubules and neurofilaments in anterior horn cells. J Cell Biol 38:224–229, 1968.
16. HIRANO A, and ZIMMERMAN HM. Some effects of vinblastine implantation in the cerebral white matter. Lab Invest 23:358–367, 1970.
17. SCHLAEPFER WW. Vincristine-induced axonal alterations in rat peripheral nerve. J Neuropathol Exp Neurol 30:488–505, 1971.
18. OWELLEN RJ, HARTKE CA, DICKERSON RM, ET AL. Inhibition of tubulin-microtubule polymerization by drugs of the vinca alkaloid class. Cancer Res 36:1499–1502, 1976.
19. DONOSO JA, GREEN LS, HELLER-BETTINGER IE, ET AL. Action of the vinca alkaloids vincristine, vinblastine, and desacetyl vinblastine amide on axonal fibrillar organelles in vitro. Cancer Res 37:1401–1407, 1977.
20. OCHS S, and WORTH RM. Comparison of the block of fast axoplasmic transport in mammalian nerve by vincristine, vinblastine and desacetyl vinblastine amide sulfate (DVA). Proc Am Assoc Cancer Res and ASCO 16:70, 1975.
21. CHAN SY, WORTH RM, and OCHS S. Block of axoplasmic transport by vinca alkaloids in desheathed nerves. (Abstr) *In* Transactions of the American Society for Neurochemistry, 1978, vol 9, p 201.
22. IQBAL Z, and OCHS S. Uptake of vinblastine, vincristine and vindesine in desheathed nerve. (Abstr) *In* Transactions of the American Society for Neurochemistry, 1978, vol 9, p 201.
23. GOTTSCHALK PG, DYCK PJ, and KIELY JM. Vinca alkaloid neuropathy: nerve biopsy studies in rats and in man. Neurology 18:875–882, 1968.
24. FOLK RM, PETERS AC, PAVKOV KL, ET AL. Vincristine (NSC-67574): a retrospective toxicologic evaluation in monkeys and dogs using weekly intravenous injections for 6 weeks. Cancer Chemother Rep Part 3, vol 5(1):17–23, 1974.
25. DYKE RW, and NELSON RL. Phase I anti-cancer agents—vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.

Cytotoxic Effects of the Lysosomotropic Agents DNA-Ellipticine, DNA-Daunorubicin, and DNA-Ellipticine:Daunorubicin on L1210 Leukemia Cells [1,2,3]

Richard A. Sorace and Bertrum Sheid [4,*]

SUMMARY

The cytotoxic effects of ellipticine, daunorubicin, and their DNA-bound lysosomotropic complexes were evaluated in L1210 leukemia grown in cell culture. Initially, it was demonstrated that these complexes were stable in a physiologic fluid environment and that metabolically unaltered drugs were released after the DNA carrier was hydrolyzed by a partially purified lysosomal fraction isolated from L1210. The 48-hour ID50 values for free and bound ellipticine were 0.20 ± 0.02 and 0.14 ± 0.02 μg/ml, respectively, while the 48-hour ID50 values for free and bound daunorubicin were both 0.026 ± 0.003 μg/ml. When compared to either of these individual complexes, the use of a single complex containing both ellipticine and daunorubicin resulted in an enhanced inhibition of cell growth and DNA and RNA synthesis. An erythrocyte ghost preparation, used to simulate a plasma membrane, was able to concentrate free but not bound ellipticine at physiologic pH. These data and our determined pK_a of ellipticine (5.8 ± 0.1) suggest that although more free ellipticine is taken up by the L1210 cells than bound ellipticine, a substantial portion of free ellipticine may be sequestered within the lipid components of the L1210 plasma membrane and thus excluded from its pharmacologically active site within the cell.

[Cancer Treat Rep 63:43–51, 1979]

The search for greater target-cell specificity in cancer chemotherapy has led to the proposal of "lysosomotropic drugs" (1,2). This approach, as described by Trouet et al (2), involves the formation of a stable complex between a suitable chemotherapeutic agent with a nonpermeant, pinocytizable, and digestible carrier, in an effort to restrict the agent's entry into malignant cells to the endocytic route. Once the complex enters the cell, the resulting phagosome will fuse with a primary lysosome and expose the carrier to the degradative actions of the lysosomal digestive enzymes. As free drug is released from the digested carrier, it could then diffuse to its pharmacologic target within the cell to exert its antineoplastic effect.

Justification for the use of the lysosomotropic approach in cancer chemotherapy has been derived from numerous reports illustrating the high endocytic activity displayed by many types of tumor cells for various macromolecules, including DNA (3–14). The actual application of this technique to cancer chemotherapy was performed using the antineoplastic antibiotic, daunorubicin, complexed with calf thymus DNA (DNA-daunorubicin) (2). Fluorescence microscopy on the exposed cells showed patterns consistent with the proposed scheme for a lysosomotropic agent. When assayed for growth-inhibitory potential on a variety of malignant cells grown in

[1]Received May 2, 1978; revised Nov 17, 1978; accepted Nov 27, 1978.

[2]Supported in part by Public Health Service grant GM-00163 from the National Institute of General Medical Sciences, National Institutes of Health, Department of Health, Education, and Welfare.

[3]This work constitutes part of a thesis submitted by R. A. Sorace in partial fulfillment of the requirements for the PhD degree at the State University of New York, Downstate Medical Center, Brooklyn.

[4]Department of Pharmacology, State University of New York, Downstate Medical Center, Brooklyn.

*_Reprint requests to:_ Dr. Bertrum Sheid, Department of Pharmacology, State University of New York, Downstate Medical Center, 450 Clarkson Ave, Brooklyn, NY 11203.

culture, DNA-daunorubicin showed cytotoxic potency comparable to that of the free drug. However, when sublethal doses of DNA-daunorubicin were given to mice bearing the L1210 leukemia, higher doses of the complex were tolerated compared to the free drug, with better therapeutic results, which were believed to be due in large part to reduced toxicity (2).

Clinical trials in patients with acute myelogenous leukemia (15) and acute nonlymphoblastic leukemia (16) demonstrated that DNA-daunorubicin was therapeutically more effective than free daunorubicin without the appearance of cardiac toxicity (a serious side effect of the free drug). Similar results illustrating the increased chemotherapeutic efficacy of a DNA-drug complex over that of the free drug in the treatment of various experimental tumors have also been demonstrated for adriamycin (17–20), ethidium bromide (21), and actinomycin D (18).

In view of the successful application of DNA-drug complexes as lysosomotropic agents, our laboratory has initiated experiments to develop new drugs and combinations of drugs which could be used effectively in their DNA-bound lysosomotropic forms. In the present study, the use of the antineoplastic alkaloid, ellipticine (22–25), complexed to a DNA carrier, and complexed to a single DNA carrier together with daunorubicin to inhibit the growth of L1210 cells grown in culture, is described.

MATERIALS AND METHODS

Materials.—Ellipticine, daunorubicin, and 1-[^{14}C]-ellipticine (specific activity, 3.17 millicuries/mmol) were generously provided to us by Dr. Harry B. Wood, Drug Development Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). Calf thymus DNA type I was purchased from Sigma Chemical Co (St. Louis, Mo). [^{3}H]-methyl-thymidine (55 curies/mmol) and [^{3}H]-5-uridine (25.6 curies/mmol) were purchased from ICN Pharmaceuticals, Inc (Irvine, Calif), while DL-1-[^{14}C]-leucine (50 millicuries/mmol) was obtained from New England Nuclear Corp (Boston, Mass). The L1210 leukemia was obtained from Dr. J. D. Broome, State University of New York, Downstate Medical Center (Brooklyn), and was carried in 20–25-g male B6D2F$_1$ mice from Jackson Memorial Laboratories (Bar Harbor, Me).

Preparation of DNA-intercalator complexes.—Ellipticine and daunorubicin have been shown to intercalate with DNA (26,27). The molar ratio of these drugs to DNA deoxynucleotides used in the following experiments has been previously shown to be in the intercalative range and essentially was not involved in other types of binding which would render the complexes unstable in physiologic media.[5] DNA was dissolved at a concentration of 2 mg/ml in 0.06 M sodium phosphate buffer, pH 7.0 (Abs_{260} = 20 for 1 mg/ml of solution). Ellipticine and [^{14}C]-ellipticine were dissolved fresh in 0.001 M HCl, while daunorubicin was dissolved fresh in the phosphate buffer. DNA-ellipticine (molar ratio of DNA mononucleotides:ellipticine of 32:1)I, DNA-[^{14}C]-ellipticine (molar ratio of DNA mononucleotides:[^{14}C]-ellipticine of 32:1)II, and DNA-daunorubicin (molar ratio of DNA mononucleotides:daunorubicin of 200:1)III or (molar ratio of DNA mononucleotides:daunorubicin of 20:1)IV, were prepared by mixing appropriate proportions of the above solutions in the phosphate buffer. DNA-ellipticine:daunorubicin (molar ratio of DNA mononucleotides:ellipticine:daunorubicin of 200:6.25:1)V or (molar ratio of DNA mononucleotides:ellipticine:daunorubicin of 200:6.25:10)VI was prepared by adding daunorubicin to 3 mg of DNA-ellipticine(I), with respect to its DNA carrier, and incubating at 37°C for 3 hours. Spectral assays were utilized to verify that the daunorubicin intercalated with the DNA without replacing any of the intercalated ellipticine.

Stability of DNA-ellipticine in a physiologic fluid environment.—The stability of the DNA-ellipticine complex was determined by allowing 0.825 μg of bound [^{14}C]-ellipticine to equilibrate with 5 ml of saline in an equilibrium dialysis cell at 37°C.[5] After 48 hours, the concentration of free [^{14}C]-ellipticine was determined using liquid scintillation spectrometry.

Lysosomal digestion of the complexes.—A lysosomal-rich subcellular fraction was isolated from L1210 cells by withdrawing 5 ml of ascites fluid from mice bearing the tumor and centrifuging them at 750 × *g* for 10 minutes. The resulting 1 ml of packed cells was resuspended in five volumes of 0.25 M sucrose (adjusted to pH 7.2). The cells were then homogenized at 0°C by eight 5-second passes with a Ten-Broeck Homogenizer attached to a Tri-R-Stir-R rotating at 12,000 rpm. Complete cell homogenization was checked after each pass by light microscopy. The whole homogenate was brought to a final volume of 6 ml with 0.25 M sucrose. One ml of homogenate was removed and frozen. The remaining 5 ml of homogenate was processed by differential centrifugation to form the following particulate fractions: [1] 850 × *g*, 10 minutes; [2] 3015 × *g*, 10 minutes; [3] 15,951 × *g*, 20 minutes; [4] 105,000 × *g*, 60 minutes; and [S] the soluble supernatant. The acid DNase activity of each fraction (thawed once)

[5]Sorace RA, and Sheid B. Characteristics of ellipticine binding to DNA. Submitted to *Chem Biol Interact.*

was determined by a previously described procedure which is based on the spectrophotometric determination of solubilized oligodeoxynucleotides (28). Protein was determined by the method of Lowry et al (29). The digestion profiles of the DNA carriers of the lysosomotropic complexes were determined in the same manner as that performed for the acid DNase assay. Thin-layer chromatography of the soluble components of the lysosomal digestion reactions was performed utilizing microcrystalline cellulose plates and a solvent system consisting of 0.1 M ethylenediamine tetraacetic acid, conc. NH_4OH, distilled water, and isobutyric acid (1.5:4:57:100) (solvent 1). In addition, 1 mg of the unbound drugs was incubated in separate lysosomal assay mixtures (28) for 2 hours at 37°C. An aliquot of supernatant containing 5 μg of drug was then spotted on microcrystalline cellulose plates and developed in an isopropanol:distilled water:HCl (65:18.5:16.5) solvent system (solvent 2). The R_f values were determined and compared to the R_f values obtained for the drugs after they had been incubated in a lysosomal-free assay mixture. The drugs were then eluted with methanol and their spectral properties were recorded.

L1210 cell culture system.—Glucose, sodium bicarbonate, penicillin, and streptomycin were added to Dulbecco's medium to give final concentrations of 3.5, 3.7, 0.1, and 0.1 g/liter, respectively. L-Asparagine was added to give a final concentration of 0.02%. The medium was filtered through a 0.22-μ Millipore filter into sterile media bottles, after which it was fortified with horse serum (10%). Inocula of washed L1210 cells (10^7) were added to flasks containing culture medium to result in a final concentration of 0.5×10^6 cells/ml, and were grown at 37°C in a 5% CO_2 atmosphere. For all experimental test procedures, 2.0-ml aliquots of the L1210 cell suspension (re-adjusted to 0.5×10^6 cells/ml) were placed in 17 × 100-mm culture tubes and grown, loosely capped, in a near-horizontal position.

Cytotoxic effects of free drugs and DNA-bound lysosomotropic complexes.—To assay cytotoxic activity, solutions of free drug or complex in 0.06 M sodium phosphate buffer were sterilized by filtering through a 0.20-μ Uni-Pore Polycarbonate Membrane. Various amounts of these agents in volumes not exceeding 0.3 ml were added to the test cultures described above. Control cultures receiving no drugs were included in all experiments. Cell counts were performed in a Coulter Counter after 24- and 48-hour incubations. The percent decrease from control cell growth was computed at each drug concentration, and the ID50 value was determined by analysis of the growth kinetics data. Pulse-labeling experiments using [^{3}H]-thymidine, [^{3}H]-uridine, and [^{14}C]-leucine were performed to examine the effects of the various complexes on DNA, RNA, and protein synthesis, respectively. This was accomplished by setting up test cultures as previously described and preincubating them for 2 hours. The cultures then received a 0.1-ml aliquot of a solution containing the appropriate labeled precursor, to give final concentrations of 1.25 microcuries/ml for either [^{3}H]-thymidine or [^{3}H]-uridine, and 0.25 microcurie/ml for [^{14}C]-leucine. To examine the time course of labeled precursor incorporation, the cells were allowed to remain in contact with the labeled material for 20-, 40-, and 60-minute periods, at which time the cultures were cooled in an ice bath and filtered through a 934 AH glass fiber filter. The filters were repeatedly washed with Ringer's solution, cold 10% trichloroacetic acid, and ethanol:ether (3:1, vol:vol). The filters were dried under a heat lamp and placed in counting vials. To compare the effects of the lysosomotropic complexes on macromolecular synthesis, the selected complex or combination of complexes was added to the test cultures at the start of the preincubation period. After 2 hours, the cultures were pulsed with the appropriate labeled precursor for 20 minutes and processed as described.

Cellular uptake of free ellipticine and DNA-ellipticine.—Uptake studies using [^{14}C]-ellipticine or DNA-[^{14}C]-ellipticine(II) were performed using 1.5 μg/ml of either free or bound [^{14}C]-ellipticine in the test cultures as previously described. The cultures were incubated for various time periods and their cell counts were determined. The cells were washed twice with Ringer's solution and dissolved in alcoholic hyamine hydroxide at 70°C. After neutralization, the solutions were added to counting vials. Uptake studies at 4°C were performed in the same manner as described above, except that the test cultures were precooled at the start of the experiment and maintained at 4°C throughout the time course shown.

Uptake of free ellipticine and DNA-ellipticine by human rbc ghosts.—Human rbc ghosts were prepared according to the method described by Steck et al (30). The uptake of [^{14}C]-ellipticine or DNA-[^{14}C]-ellipticine(II) by the rbc membranes was examined using a modification of the method described by Kang and Lee (31). Rbc ghost suspensions (100 μg of protein) were plated on Corning microscope cover glasses. After drying them overnight in a dessicator, they were equilibrated for 10 minutes with 5 mM sodium phosphate buffer, pH 7.0. These preparations were then incubated with 2 μg/ml of either free or bound [^{14}C]-ellipticine in the sodium phosphate buffer for varying time periods at 22°C. The membrane preparations were then washed by sequentially dipping each cover glass into five beak-

TABLE 1.—Intracellular distribution of acid DNase

Parameter assayed	Absolute values for homogenate*	% homogenate fractions†					% recovery
		[1]	[2]	[3]	[4]	[S]	
Protein	102	22.1	12.0	7.7	18.4	38.4	98.6
Acid DNase	0.0226	0	30.4	51.8‡	0	17.3	99.5

*Absolute values are mg for protein and total units of activity for acid DNase (1 unit = 1 μmol of liberated mononucleotides/min).

†Fractions: [1] 850 × *g*, 10 mins; [2] 3015 × *g*, 10 mins; [3] 15,951 × *g*, 20 mins; [4] 105,000 × *g*, 60 mins; and [S] soluble supernatant.

‡Alkaline DNase specific activity was 0.323 unit/g of protein in contrast to an acid DNase specific activity of 1.48 units/g of protein.

ers, each containing 200 ml of buffer. The cover glasses were dried under a heat lamp and placed into counting vials.

RESULTS

Complex stability in a physiologic fluid environment.—Previous experiments in our laboratory[6] demonstrated that the physicochemical properties of the DNA-ellipticine intercalative complex are in accord with the criteria necessary for a useful lysosomotropic agent. Briefly, it was demonstrated that ellipticine intercalates with DNA up to 1 mol of drug/13 mols of DNA deoxynucleotides by means of hydrophobic and hydrogen binding. This type of binding affords the DNA-drug complex stability in a physiologic media. Higher amounts of ellipticine surfaced bound to DNA by mainly electrostatic binding to the sugar phosphate backbone of DNA. This type of binding proved unstable in physiologic media. Therefore, the molar ratio of drug to DNA deoxynucleotides used in these experiments was in the intercalative region. This allows DNA-bound drugs, rather than dissociated free drugs, to enter the cells. A direct assessment of the stability of the DNA-ellipticine complex utilizing radioactive drug in an equilibrium dialysis cell[6] revealed that in a physiologic medium (saline or sera), at 37°C, < 7% of the bound ellipticine was released during a 48-hour time period.

Lysosomal digestion of the DNA-drug complexes.—Since the DNA carriers must eventually be hydrolyzed by the L1210 cell's lysosomal acid DNase, acid DNase was used as a marker enzyme for the preparation of a lysosomal-enriched subcellular fraction. Data presented in table 1 summarize the intracellular distribution of acid DNase activity in the L1210 leukemia. The percentage of the homogenate's total acid DNase activity in fractions [2] and [3] was 30.4% and 51.8%, respectively, with 17.3% of the activity in the soluble supernatant fraction, and no significant activity in fractions [1] and [4]. The specific activity in fractions [2] and [3] represented a 2.5- and 6.7-fold purification, respectively, over that of homogenate. The specific activity of the DNase under alkaline conditions was 21.8% of the specific activity under acidic conditions. Consequently, the majority of the DNase activity can be attributed to the presence of acid DNase in a lysosomal-rich fraction, and not the tail end of an alkaline DNase. Figure 1 shows that when DNA and the various DNA-drug complexes were incubated in vitro with the partially purified lysosomal fraction, DNA and DNA-ellipticine(I) displayed some initial hydrolysis after 1 hour, while DNA-daunorubicin(IV) and DNA-ellipticine:daunorubicin(VI) were essentially resistant to hydrolysis for a 2-hour time period. DNA and DNA-ellipticine displayed identical enzymatic digestion profiles, and reached 100% digestion after 48 hours. Although the DNA-daunorubicin digestion was slower than the digestion of the previous two complexes, it also reached 100% digestion after 48 hours. DNA-ellipticine:daunorubicin displayed the most resistance to digestion, and reached 95% digestion of the carrier after 48 hours. In all cases the rate of digestion was greatest during the initial 24-hour period. Thin-layer chromatograms of the soluble components of the lysosomal digestion reactions using solvent 1 displayed a complete separation of the four major bases of DNA and the free drugs (both free drugs having R_f values of 0.95). No DNA fragments appeared after lysosomal digestion other than the deoxymononucleotides. In addition, when standard samples of the free drugs were incubated in a lysosomal assay mixture, in a lysosomal-free assay mixture, or in methanol, they had the same R_f values when developed in solvent 2 (ellipticine, 0.56,

[6]See footnote 5.

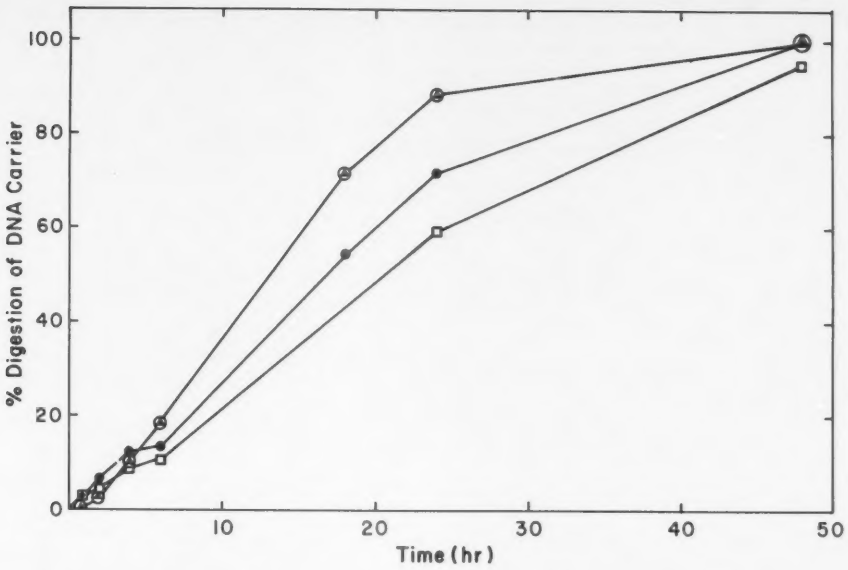


FIGURE 1.—Lysosomal digestion of DNA-drug complexes. % digestion of the DNA carriers by an extract of a partially purified lysosomal fraction isolated from the L1210 leukemia is shown for native DNA (○——○), DNA-ellipticine(I) (△——△), DNA-daunorubicin(IV) (●——●), and DNA-ellipticine:daunorubicin(VI) (□——□).

and daunorubicin, 0.76). After elution with methanol, lysosomal-exposed and nonexposed drugs displayed identical spectral properties (absorption maximum of ellipticine, 305 nm, and daunorubicin, 480 nm).

Cytotoxic effect of free drugs and DNA-bound lysosomotropic complexes.—The ID50 values for free drugs and DNA-bound drugs were determined in L1210 leukemia grown in log-phase cell culture. Table 2 summarizes the ID50 values obtained from the analysis of the growth kinetics for all of the agents screened. The ID50 value at 48 hours for free ellipticine was 0.20 ± 0.02 μg/ml, while the ID50 value at 48 hours for DNA-ellipticine(I) was 0.14 ± 0.02 μg/ml with respect to the bound drug. At 24 and 48 hours, equivalent cytotoxic effects were observed between the complex and free drug when the DNA-bound ellipticine concentration was approximately 30% less than that of the free ellipticine. The ID50 values at 48 hours for free daunorubicin and DNA-daunorubicin(III) were both 0.026 ± 0.003 μg/ml with respect to the daunorubicin concentration. A comparison of the cytotoxic effects produced by using a single DNA carrier prepared with both ellipticine and daunorubicin with either DNA-ellipticine or DNA-daunorubicin is also presented in table 2. The ID50 value at 48 hours for DNA-ellipticine:daunorubicin(V) was 0.083 ± 0.011 and 0.021 ± 0.003 μg/ml with regard to bound ellipticine and daunorubicin, respectively. Table 2 also shows that the ID50 value of DNA-daunorubicin(IV) was 0.028 ± 0.004 μg/ml with respect to bound daunorubicin, which is in close agreement with the ID50 value determined for free daunorubicin. The effect of increasing the daunorubicin content of the complex tenfold resulted in a near-tenfold decrease in the concentration of DNA carrier necessary for

TABLE 2.—ID50 values for free ellipticine, free daunorubicin, and their DNA-bound lysosomotropic complexes*

Drug	ID50 values (48 hrs)	
	Ellipticine† (μg/ml)	Daunorubicin‡ (μg/ml)
Free ellipticine§	0.20 ± 0.02	—
DNA-ellipticine(I)¶	0.14 ± 0.02	—
Free daunorubicin	—	0.026 ± 0.003
DNA-daunorubicin(III)	—	0.026 ± 0.003
DNA-ellipticine:daunorubicin(V)	0.083 ± 0.011	0.021 ± 0.003
DNA-daunorubicin(IV)**	—	0.028 ± 0.004

*All of the given values were calculated for each agent screened by using the growth kinetics obtained from a series of increasing drug concentrations with 8 test cultures used at each drug level.

†Concentration of either free or bound ellipticine.

‡Concentration of either free or bound daunorubicin.

§24-hr ID50 value was 0.44 ± 0.06 μg/ml.

¶24-hr ID50 value was 0.29 ± 0.04 μg/ml.

**Antitumor effectiveness of DNA-ellipticine/daunorubicin(VI) was not significantly different from DNA-daunorubicin(IV).

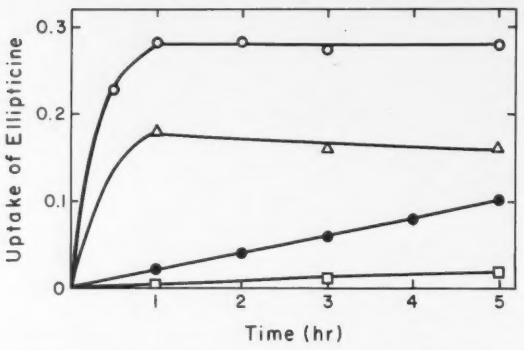


FIGURE 2.—Cellular uptake of free ellipticine and DNA-ellipticine. The uptake of ellipticine is expressed as μg of drug/10^6 cells for free ellipticine at 37°C (○——○) and 4°C (△——△) and bound ellipticine at 37°C (●——●) and 4°C (□——□).

delivering equi-effective drug amounts capable of producing 50% growth inhibition. It should be noted that this higher-bound daunorubicin complex was more than ten times more potent than the DNA-ellipticine complex studied. Consequently, the use of DNA-ellipticine:daunorubicin(VI) did not result in a dramatic increase in the complex's effectiveness over that of DNA-daunorubicin(IV). The addition of equivalent amounts of DNA in the absence of bound drugs produced negligible to slight stimulatory effects on the control log-phase growth curve.

Efficacy of using a single DNA carrier with two bound drugs.—The efficacy of using DNA-ellipticine:daunorubicin(V) over either DNA-ellipticine(I), DNA-daunorubicin(III), or a combination of both complexes used together, is illustrated in table 3. DNA-ellipticine, by itself, inhibited both DNA and RNA synthesis, whereas DNA-daunorubicin preferably inhibited RNA synthesis. When compared to either of these individual complexes, DNA-ellipticine:daunorubicin demonstrated an enhanced inhibition of both DNA and RNA synthesis and cell growth. The administration of the bound drugs as DNA-ellipticine:daunorubicin, compared to an equivalent amount of bound drugs as DNA-ellipticine plus DNA-daunorubicin, also resulted in a significantly higher inhibition of cell growth, DNA synthesis, and RNA synthesis. Significant differences in the inhibition of protein synthesis were not apparent.

Cellular uptake of free ellipticine and DNA-ellipticine.—Figure 2 shows the uptake of equivalent concentrations of free and bound [^{14}C]-ellipticine by L1210 cells. The amount of free ellipticine in association with 10^6 cells at 37°C increased rapidly up to a plateau level in 1 hour, and remained at this level over the remaining 5-hour time course. In contrast, bound ellipticine in association with 10^6 cells at 37°C increased in a slow linear manner over the 5-hour period studied. Measurements performed up through 48 hours showed approximately a 50% increase of free ellipticine uptake in comparison to the DNA-bound complex. It should be noted, however, that the high degree of cytotoxicity for both these agents at longer contact times made it difficult to determine precise values of cellular uptake. When the experiment was performed at 4°C, the uptake of

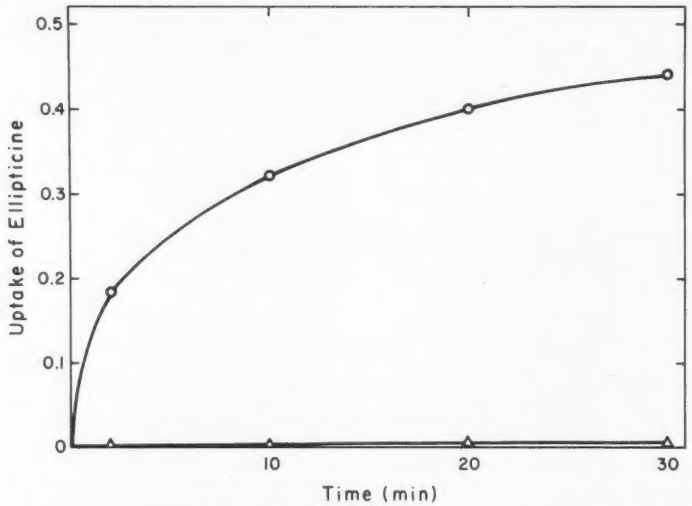


FIGURE 3.—Uptake of free ellipticine and DNA-ellipticine by human rbc ghosts. The uptake of ellipticine is expressed as μg of drug/100 μg of rbc ghost protein for free ellipticine (○——○) and bound ellipticine (△——△) at 22°C.

TABLE 3.—Effects of lysosomotropic complexes on cell growth and macromolecular synthesis*

Parameter	% inhibition from controls for:†			
	DNA-ellipticine: daunorubicin	DNA-ellipticine + DNA-daunorubicin	DNA-ellipticine	DNA-daunorubicin
Cell growth	46.0 ± 0.6‡	35.0 ± 1.0‡	31.0 ± 0.06	32.0 ± 0.8
DNA synthesis	21.0 ± 2.2§	12.6 ± 0.6§	18.9 ± 2.1	7.8 ± 2.8
RNA synthesis	39.5 ± 1.1¶	27.8 ± 0.7¶	26.0 ± 0.04	30.0 ± 2.2
Protein synthesis	31 ± 6	29 ± 3	25 ± 5	32 ± 5

*The complexes DNA-ellipticine(I), DNA-daunorubicin(III), and DNA-ellipticine:daunorubicin(V) were each incubated at a concentration of 6.8 μg/ml with respect to their DNA carriers with the L1210 leukemia. Cell growth was determined after 24 hrs and macromolecular synthesis after 2 hrs. The incorporation of all labeled precursors was linear over a 60-min time course. Significance levels for the comparison of selected means were determined by a 2-tailed *t* test. The results for free ellipticine and free daunorubicin were similar to those of the DNA-bound drugs.

†All assays were performed in quadruplicate on 4 different occasions.

‡$P < 0.001$.

§$P < 0.02$.

¶$P < 0.01$.

bound ellipticine still increased in a slow linear manner; however, the uptake was greatly reduced. The uptake of free ellipticine at 4°C still proceeded, with a rapid increase up to a plateau level in 1 hour; however, the height of the plateau was reduced compared to the level at 37°C.

Nonspecific plasma membrane accumulation of free ellipticine.—The pK_a of ellipticine was determined to be 5.8 ± 0.1. Consequently, at physiologic pH, approximately 98% of the ellipticine molecules will be in their uncharged form and will display a highly lipophilic character. In view of past reports suggesting that ellipticine will concentrate in lipid phases at physiologic pH (22,27), it appeared plausible that the substantial increment of free ellipticine taken up by the L1210 cells represents free drug that has become dissolved in the lipid components of the plasma membrane. To provide a model system to test the assumption that free ellipticine will become concentrated within the plasma membrane at physiologic pH, the uptake of equivalent concentrations of free and bound [^{14}C]-ellipticine by human erythrocyte ghosts was studied. Figure 4 shows the uptake of free and DNA-bound [^{14}C]-ellipticine at 22°C over a 30-minute time course. The uptake of free ellipticine was most rapid in the initial few minutes and reached 0.45 μg/100 μg of membrane protein by 30 minutes. In sharp contrast, the uptake of DNA-ellipticine was essentially negligible throughout the 30-minute incubation period.

DISCUSSION

The complex formed between native calf thymus DNA and ellipticine fulfilled the criteria for a useful lysosomotropic agent. The complex was shown to be essentially stable within a physiologic fluid environment, and the DNA carrier was digestible by the target cells' lysosomal acid DNase. Chromatographic and spectral analysis of the lysosomal digestion products revealed that the released drugs did not undergo any chemical alterations during their enzymatic release process or passage through the lysosomal environment. These data indicate that in an intact cell system the drug would now be ready to leave the lysosome in its biologically active form and exert its pharmacologic effect within the cell.

The lysosomotropic complexes proved to be effective as cytotoxic agents in inhibiting the L1210 cells' log-phase growth in a dose-dependent manner. DNA-ellipticine was more effective than free ellipticine in suppressing cell growth, while DNA by itself had essentially no effect on the growth of the L1210 cells (tables 1 and 2). These data indicate that the complex rather than free drug (dissociated from the complex) is the major uptake species when the complex is used. Similar conclusions can be inferred from the experiments of other investigators (15,16) who demonstrated that a DNA-daunorubicin complex was at least as effective as free daunorubicin in treating human leukemia. Since the complex did not produce the characteristic cardiotoxicity of free daunorubicin, it is reasonable to assume that the complex, and not the free drug (dissociated from the complex), was the major uptake species when the complex was employed. The use of a complex prepared with both ellipticine and daunorubicin produced a greater cytotoxic effect than either DNA-ellipticine(I) or DNA-daunorubicin(III) used separately. This increased efficacy of DNA-

ellipticine:daunorubicin(V) may have occurred in part because these two drugs inhibit cell growth by different mechanisms of action. Although both agents intercalate with DNA, daunorubicin preferentially inhibits RNA synthesis (26,32,34), while ellipticine inhibits DNA and RNA synthesis (24,33,34). Ellipticine also has a direct effect on the processing of 45s nucleolar RNA, while daunorubicin has no effect on the processing mechanism (35). In addition, daunorubicin, unlike ellipticine, produces a moderate reduction in the chain length of nucleoplasmic RNA and a marked inhibition of nucleolar 45s RNA synthesis (36).

The administration of equivalent amounts of the bound drugs in the form of either DNA-ellipticine:daunorubicin(V) or DNA-ellipticine(I) plus DNA-daunorubicin(II) resulted in significantly different inhibitory effects on cell growth, DNA synthesis, and RNA synthesis, with complex V being more effective in all cases. The results illustrate the efficacy of using a single carrier with two drugs, as opposed to using the same amount of both drugs on separate carriers. When considered within the context of the mechanism of drug uptake, the results provide a supportive argument for a carrier-mediated endocytic mechanism, as opposed to the process of free-drug diffusion across the plasma membrane from a dissociated complex. If the latter mechanism were operative, the inhibitory values produced by the two modes of bound-drug administration would have been expected to be equivalent.

The kinetics of free and bound ellipticine uptake by the L1210 cells was similar to the results reported for the uptake of free and bound daunorubicin by L1210 cells (37). The uptake of DNA-ellipticine displayed a temperature dependence analogous to that of DNA-daunorubicin. This marked temperature dependence for complex uptake is consistent with an endocytic mechanism, since such a process is temperature-sensitive and energy-dependent (11). The partial temperature dependence of free ellipticine uptake is in contrast to that of free daunorubicin, since low temperatures prevented drug uptake in the latter case. Since carrier-mediated membrane transport, in contrast to diffusion, is generally expected to have a high degree of temperature dependence, the uptake of free ellipticine may be mediated in part by a specific membrane carrier. The alternative possibility of a diffusion system with a high degree of temperature dependence cannot be ruled out at this time. The mechanism of free daunorubicin uptake has been attributed to either a specific membrane carrier or a temperature-sensitive diffusion process (38).

That free ellipticine is taken up by the L1210 cells to a greater extent than bound ellipticine, yet requires a higher concentration than bound ellipticine for 50% growth inhibition, suggests that a substantial portion of the free ellipticine molecules taken up by the cells may be excluded from interacting with their pharmacologic target within the cell. This may be due to the equilibrium of free drug between various intracellular and extracellular compartments. An alternate explanation may be suggested from previous reports showing that free ellipticine will concentrate in lipid phases at physiologic pH (22,27), which is consistent with our measured pK_a of ellipticine (5.8 ± 0.1). Based on these latter data, it can be stated that, at physiologic pH, approximately 98% of the ellipticine molecules will be in their uncharged form and highly lipophilic. Therefore, we are suggesting that the substantial portion of free ellipticine molecules being excluded from their target within the cell represents free drug that specifically has become sequestered within the lipid components of the cell's plasma membrane. This assumption was tested using an erythrocyte ghost preparation to simulate a pure plasma membrane system. Erythrocyte ghosts were chosen since they can be quantitatively prepared free of contamination. The results demonstrated that the plasma membrane possesses the ability to concentrate free, but not bound, ellipticine at physiologic pH. If the plasma membrane of the L1210 cell is acting in a similar manner, it could act as a sequestration site to prevent free ellipticine internalization, since ellipticine molecules dissolved within its lipid phase would be effectively excluded from their pharmacologically active site within the cell. Hence, the administration of ellipticine in its DNA-bound lysosomotropic form may circumvent this problem of nonspecific accumulation within the plasma membrane and provide a more direct route for the entry of drug inside the cell, thus resulting in an ultimate enhancement of its cytotoxic effect.

REFERENCES

1. DE DUVE C, DE BARSY T, POOLE B, ET AL. Commentary: Lysosomotropic agents. Biochem Pharmacol 23:2495–2531, 1974.
2. TROUET A, DEPREZ-DE CAMPENEERE D, and DE DUVE C. Chemotherapy through lysosomes with a DNA-daunorubicin complex. Nature [New Biol] 239:110–112, 1972.
3. ADAMS JE, MARTIN WE, and POMERAT CM. Incorporation of deoxyribonucleic acid by HeLa cells during various phases of the cell cycle. Tex Rep Biol Med 23:191–203, 1965.
4. BENSCH KG, and KING DW. Incorporation of heterologous deoxyribonucleic acid into mammalian cells. Science 133:381–382, 1961.
5. BENSCH K, GORDON G, and MILLER L. The fate of DNA-containing particles phagocytized by mammalian cells. J Cell Biol 21:105–114, 1964.
6. BUSCH H, FUJIWARA E, and FIRSZT DC. Studies on the

metabolism of radioactive albumin in tumor-bearing rats. Cancer Res 21:371–377, 1961.
7. CAULFIELD JB. Studies on ferritin uptake by isolated tumor cells. Lab Invest 12:1018–1025, 1963.
8. COHN ZA, and PARKS E. The regulation of pinocytosis in mouse macrophages. J Exp Med 125:213–229, 1967.
9. EASTON JM, GOLDBERG B, and GREEN H. Demonstration of surface antigens and pinocytosis in mammalian cells with ferritin-antibody conjugates. J Cell Biol 13:437–443, 1962.
10. GEY GO. Some aspects of the constitution and behavior of normal and malignant cells maintained in continuous culture. Harvey Lect 50:154–229, 1956.
11. GLICK JL. Quantitative studies concerning the uptake of mouse thymus DNA by L1210 leukemia cells. Cancer Res 27:2350–2356, 1967.
12. HANDLER ES, HANDLER EE, and CHESEBROUGH AB. Phagocytic activity of leukemic myeloblasts. J Reticuloendothel Soc 6:511–524, 1969.
13. MEGO JL, and MCQUEEN JD. The uptake of labeled proteins by particulate fractions of tumor and normal tissues after injection into mice. Cancer Res 25:865–869, 1965.
14. RYSER HJP. Uptake of proteins by mammalian cells: an undeveloped area. Science 159:390–396, 1968.
15. SOKAL G, TROUET A, MICHAUX JL, ET AL. Letter: DNA-daunorubicin complex: preliminary trials in human leukaemia. Eur J Cancer 9:391–392, 1973.
16. CORNU G, MICHAUX J-L, SOKAL G, ET AL. Daunorubicin-DNA: further clinical trials in acute non-lymphoblastic leukemia. Eur J Cancer 10:695–700, 1974.
17. ATASSI G, and TAGNON HJ. Comparison of adriamycin with the DNA-adriamycin complex in chemotherapy of L1210 leukemia. Eur J Cancer 10:399–403, 1974.
18. MARKS TA, and VENDITTI JM. Potentiation of actinomycin D or adriamycin antitumor activity with DNA. Cancer Res 36:496–504, 1976.
19. ROZENCWEIG M, KENIS Y, ATASSI G, ET AL. DNA-adriamycin complex: preliminary results in animals and man. Cancer Chemother Rep Part 3, vol 6(2):131–136, 1975.
20. TROUET A, DEPREZ-DE CAMPENEERE D, DE SMEDT-MALENGREAUX M, ET AL. Experimental leukemia chemotherapy with a "lysosomotropic" adriamycin-DNA complex. Eur J Cancer 10:405–411, 1974.
21. HEINEN E, BASSLEER R, CALBERG-BACQ CM, ET AL. Comparative study of the effects of ethidium bromide and DNA-ethidium bromide complex on normal and cancer cells. Biochem Pharmacol 23:1549–1551, 1974.
22. HARDESTY CT, CLANEY NA, and MEAD JAR. The effect of route of administration on the distribution of ellipticine in mice. Cancer Res 32:1884–1889, 1972.
23. HARTWELL JL, and ABBOTT BJ. Antineoplastic principles in plants: recent developments in the field. Adv Pharmacol 7:117–209, 1969.
24. LI LH, and COWIE CH. Biochemical effects of ellipticine on leukemia L1210 cells. Biochim Biophys Acta 353:375–384, 1974.
25. SVOBODA GH, POORE GA, and MONTFORT ML. Alkaloids of *Ochrosia maculata Jacq.* (*Ochrosia borbonica Gmel.*). Isolation of the alkaloids and study of the antitumor properties of 9-methoxyellipticine. J Pharm Sci 57:1720–1725, 1968.
26. DI MARCO A. Adriamycin (NSC-123127): mode and mechanism of action. Cancer Chemother Rep Part 3, vol 6(2):91–106, 1975.
27. KOHN KW, WARING MJ, GLAUBIGER D, ET AL. Intercalative binding of ellipticine to DNA. Cancer Res 35:71–76, 1975.
28. BARRET AJ. Lysosomal hydrolyases. *In* Lysosomes—A Laboratory Handbook (Dingle JT, ed). New York, American Elsevier Publishing Co, 1972, p 114.
29. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275, 1951.
30. STECK TL, WEINSTEIN RS, STRAUSS JH, ET AL. Inside-out red cell membrane vesicles: preparation and purification. Science 168:255–257, 1970.
31. KANG DH, and LEE KS. A modified method for studying the interaction of isolated cardiac cell membrane with ions and drugs. Anal Biochem 75:345–355, 1976.
32. BARBIERE P, DI MARCO A, MAZZOLENI R, ET AL. Azione della daunomicina sul metabolismo degli acidi nucleinici nell'*E. coli* B. G Microbiol 12:71–82, 1974.
33. KERSTEN W, KERSTEN H, and SZYBALSKI W. Physicochemical properties of complexes between deoxyribonucleic acid and antibiotics which affect ribonucleic acid synthesis. Biochemistry 5:236–244, 1966.
34. SORACE RA, and SHEID B. Antitumor effects of DNA-ellipticine and DNA-ellipticine:daunorubicin on the L1210(A) leukemia. Fed Proc 36:349, 1977.
35. SNYDER AL, KANN HE, and KOHN KW. Inhibition of the processing of ribosomal precursor RNA by intercalating agents. J Mol Biol 58:555–565, 1971.
36. KANN HE, JR, and KOHN KW. Effects of deoxyribonucleic acid-reactive drugs on ribonucleic acid synthesis in leukemia L1210 cells. Mol Pharmacol 8:551–560, 1972.
37. OHNUMA T, HOLLAND JF, and CHEN J-H. Pharmacological and therapeutic efficacy of daunomycin:DNA complex in mice. Cancer Res 35:1767–1772, 1975.
38. DANØ K. Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. Biochim Biophys Acta 323:466–483, 1972.

Pharmacology and Toxicology of α-2′-Deoxythioguanosine in Cancer Patients [1,2]

G. A. LePage [3,*] and A. Khaliq [4]

SUMMARY

α-2′-Deoxythioguanosine (α-TGdR) was administered as a single dose to 13 cancer patients in 18 experiments at dose levels of 150–1500 mg/m² and as a daily dose to 22 patients in 42 experiments at dose levels of 100–4000 mg/m²/day × 5 days. No significant toxicity was observed. Blood levels and rates of excretion were determined with radiosulfur-labeled α-TGdR. Approximately 80% of the dose was excreted in the urine in 24 hours, initially as unchanged α-TGdR and increasingly as metabolites. Metabolites appear to be nucleosides and do not include 6-thioguanine, 6-thioxanthine, or 6-thiouric acid to any measurable extent. Small amounts of the α-TGdR in blood samples were bound to albumin and to erythrocyte membranes. Blood plasma levels of α-TGdR at the highest doses were in the range of 200–300 μM, declining in 24 hours to 67–124 μM.

[Cancer Treat Rep 63:53–57, 1979]

α-2′-Deoxythioguanosine (α-TGdR) became available incidental to the chemical synthesis of β-2′-deoxythioguanosine (β-TGdR) (1). It was found to be active against several experimental neoplasms in mice (2) including the L1210 leukemia, with little or no toxicity to the mice at effective doses. Subsequent studies have shown that α-TGdR was phosphorylated by a kinase(s) present in affected experimental neoplasms (3) but not present in normal tissues. Surveys (4,5) indicated that this phosphorylative activity was present in varying amounts in an appreciable percentage of human tumors, but again not in the normal tissues. When the kinase was present in mouse tumors, α-TGdR was phosphorylated to the triphosphate level and incorporated into DNA in terminal position (3). This has been demonstrated to result in the accumulation in affected cells of short DNA chains (6). Some of the properties of the kinases have been studied (5). The toxicology of α-TGdR in dogs and monkeys has been reported (7). Toxic effects were obtained only at very high doses. Lethal effects were produced in two dogs, at doses of 2000 and 4000 mg/m²/day × 5 days, respectively, whereas no lethal effects were obtained in the treatment of monkeys with doses of 7200 mg/m²/day × 5 days. We have now conducted tests on cancer patients with single doses of 150–1500 mg/m² and with daily doses of 100–4000 mg/m²/day × 5 days without any significant toxicity. Plasma levels and urinary excretion have been monitored in each case by the use of a radiosulfur label in the α-TGdR.

MATERIALS AND METHODS

α-TGdR was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, Md), in sterile vials as a lyophilized material containing 200 mg of α-TGdR, 10 mg of mannitol, and NaOH so that on reconstitution with water to 20.0 ml the pH was approximately 10. Three different lots of formulated material were used. These were assayed for β-TGdR content by labeling with ^{35}S by exchange, incubating with an anomerically specific purine nucleoside phosphorylase (Sigma Chemical Co, St. Louis, Mo), chromatographing on Whatman

[1]Received May 23, 1978; revised Nov 7, 1978; accepted Nov 27, 1978.

[2]Supported by the National Cancer Institute of Canada and the Alberta Provincial Cancer Hospitals Board.

[3]Cancer Research Unit, McEachern Laboratory, and Department of Biochemistry, University of Alberta, Edmonton, Canada.

[4]Cross Cancer Institute, Edmonton, Alberta, Canada.

Reprint requests to: Dr. G. A. LePage, McEachern Laboratory, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

3MM papers, and measuring radioactivity found in 6-thioguanine (6-TG) (3). The three lots contained 0.35%, 1.00%, and 1.14% of β-TGdR, respectively. α-TGdR free of β-TGdR was prepared and labeled with ^{35}S by exchange with rhombic ^{35}S (New England Nuclear Corp, Boston, Mass) as described earlier (3). ^{35}S-α-TGdR was added to the α-TGdR solutions to be used for patients, in appropriate amount, usually 100 microcuries when a single dose was being studied. When 5-day courses were being monitored, the amount of ^{35}S-α-TGdR was reduced to approximately 20 microcuries/dose. Informed consent was obtained from the patients. The α-TGdR solutions, 10 mg/ml, were administered iv through Swinnex 25 filters (Millipore Corp, Bedford, Mass). Blood samples were drawn into heparinized tubes at intervals thereafter and immediately chilled. Urine samples were collected and stored at 0°C–2°C until analyzed. Blood samples were routinely centrifuged, an aliquot of plasma was drawn off, and the plasma was treated with an equal volume of 0.4 M perchloric acid. Protein was centrifuged and extracts were neutralized to pH 6 with KOH. Aliquots of neutralized extracts and of urine samples (250 μl) were counted in a scintillation system and further aliquots were chromatographed on Whatman 3MM paper sheets with three descending solvent development systems. These were: (*a*) 5% KH_2PO_4 in water, (*b*) 95% ethanol:1 M ammonium acetate in water (5:2, vol:vol), and (*c*) butanol:acetic acid:H_2O (120:30:50, vol:vol). In some instances, urine obtained before the dose of α-TGdR was used to dissolve marker compounds since this influenced R_f values. Marker compounds used were α-TGdR, 6-thioxanthine (6-TX), 6-thiouric acid (6-TU), 6-TG, and 6-methylthioguanine. Spots from the dried papers were cut out and counted directly in a polar scintillation medium (8) that gave counts equal to those obtained with an equivalent radioactivity in solution.

In several instances, heparinized whole blood samples were taken soon after (1 hour) the administration of ^{35}S-α-TGdR, before metabolites were evident, and the cells were lysed by the addition of 3 volumes of water; aliquots therefrom were used for electrophoresis on polyacrylamide gels carried out by standard procedures to separate the proteins and determine whether ^{35}S-α-TGdR was bound to any macromolecular component. Gels were sliced and slices (1 mm) oxidized with hydrogen peroxide in scintillation counting vials. Samples were then subjected to scintillation counting and matches were made with the locations of the protein components. This was also carried out with a sample of whole blood (from an untreated individual) which was mixed in vitro at 37°C for 1 hour with a 100 μM concentration of ^{35}S-α-TGdR.

Since this was a phase I study, no attempt was made to select patients whose tumors had the capacity to activate the drug (α-TGdR kinase) (4). Patients were treated as they became available. Three patients were evaluated at each dose level before escalation to the next dose level. Patients were treated with single doses at 150, 300, 450, 600, 1000, and 1500 mg/m^2. Daily doses for 5 successive days were then given to groups of three patients at 100, 150, 225, 340, 510, 750, 1000, 1250, 2000, 2500, and 4000 mg/m^2. The two highest doses were divided, with half given in the morning and the other half given 6–8 hours later. Some patients received a second treatment 1 month later at a higher dose level. A total of 35 patients received 60 doses of ^{35}S-α-TGdR. All had normal kidney, liver, and bone marrow function. Their diagnoses were as follows: melanomas, eight patients; stomach carcinomas, seven; colon carcinomas, eight; carcinoma of the pancreas, two; acute myelogenous leukemia, two; chronic myelogenous leukemia in blastic crisis, one; Hodgkin's disease, one; prolymphocytic leukemia, one; breast carcinoma, one; renal carcinoma, one; adenoid cystic carcinoma, one; adenocarcinoma of unknown primary, one; and squamous cell carcinoma of unknown primary, one. Some patients had received radiotherapy. Three patients had had no prior treatment.

RESULTS

Clinical Evaluation

With doses of 2500 and 4000 mg/m^2/day, patients complained of nausea during the injections. There were no other significant side effects. None of the patients showed any abnormality on urinalysis or hepatic and renal function tests. With one exception, there were no changes in blood cell counts. Chest roentgenograms showed no infiltrates related to the drug treatments. There were mild "recall phenomena" (mild erythema of the skin over the irradiated area) shown by patients who had received prior radiation therapy.

One patient with metastatic carcinoma of the colon was found to have a viral infection. During treatment with α-TGdR at 1000 mg/m^2 × 5 days, she had transient leukopenia (1.8 × 10^3 cells/mm^3) and thrombocytopenia (75 × 10^3 cells/mm^3).

None of the patients showed a significant response but, as indicated, these patients were not selected for tumors with capacity to activate the drug.

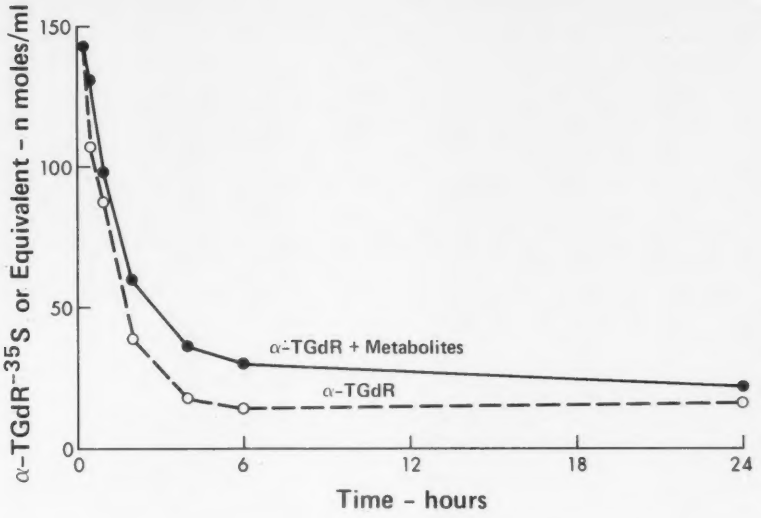


FIGURE 1.—Plasma levels of α-TGdR and metabolites. Patient 1 was given a total iv dose of 1500 mg of ^{35}S-α-TGdR (1000 mg/m^2). Plasma samples were deproteinized and chromatographed to separate ^{35}S-α-TGdR and metabolites.

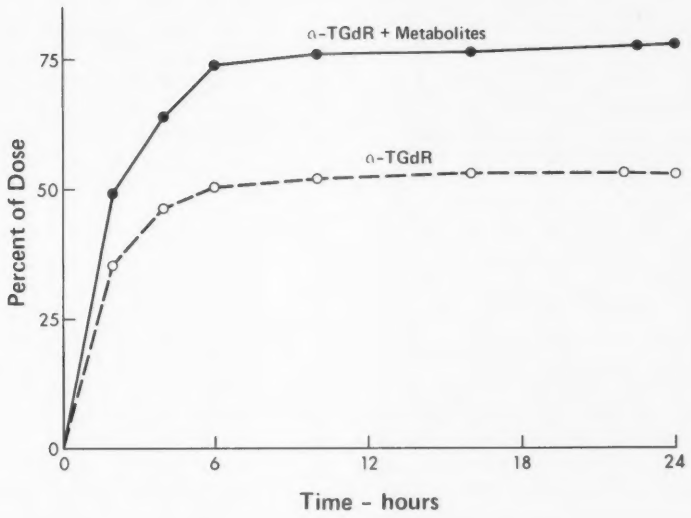


FIGURE 2.—Urinary excretion of α-TGdR and metabolites. Data are from the same experiments as shown in fig 1.

TABLE 1.—Analyses for plasma levels of α-TGdR after iv administration of indicated doses*

Daily dose (mg/m²)	Levels of α-TGdR† (μM)									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	30 mins	24 hrs	30 mins	24 hrs	30 mins	24 hrs	30 mins	24 hrs	30 mins	24 hrs
100	11.3	0.7	11.7	0.8	11.1	1.0	13.8	—	13.9	4.9
150	28.0	3.1	22.5	3.0	19.5	3.5	21.4	4.0	20.2	5.3
225	40.8	9.3	42.2	11.9	34.0	7.2	36.2	8.1	46.4	7.9
340	64.4	5.8	63.9	8.7	62.3	4.9	63.9	20.1	87.7	20.1
510	47.3	4.7	46.8	6.1	48.2	5.6	51.3	6.1	53.8	6.5
750	83.0	4.7	100.7	8.2	120	5.1	119	5.3	66.8	4.2
1000	154	17.3	143	32.5	137	4.1	167	26.8	115	18.2
1250	111	5.2	—	—	—	—	—	—	—	—
1500	139	105	183	65.3	195	121	219	121	310	109
2000	229	54.3	240	54.3	236	66.0	248	64.6	231	73.0
2500	206‡	90	198	88	225	94	156	113	192	124
	229	90	220	121	208	93	218	67	223	89
4000	139‡	69	219	48	261	98	241	83	216	99
	204	85	252	116	301	96	303	79	300	77

* Measurements were made on perchloric acid extracts of plasma.
† In most instances each figure is an average of analyses from 3 patients.
‡ Divided doses (ie, 1250 and 2000 × 2 doses): Nos. given are for 30 mins after dose and just prior to next dose.

When blood proteins exposed to ^{35}S-α-TGdR were separated on polyacrylamide gels, no radioactivity was associated with the globulins or the hemoglobin. The albumin fraction contained 6.9% of the radioactivity, and the "cell ghosts" contained 5.3%. Thus, binding was minimal, at least at the time monitored (1 hour).

For the highest doses given (2500 and 4000 mg/m²/day × 5 days), lot No. 73–223 was used. This lot contained 1.14% of β-TGdR and it is significant that this level of contamination did not appear to have any deleterious effects.

Metabolism and Excretion

Data are presented in figure 1 for the blood levels of α-TGdR after iv administration of a single dose at 1000 mg/m². This is representative of the findings. The patient (No. 1) had a body surface area of 1.5 m² and received a total dose of 1500 mg of α-TGdR. The initial plasma concentration was 143 μM and this declined to approximately 17 μM by 6 hours. Urinary excretion in 24 hours was 78.3% of the dose, predominantly as unchanged α-TGdR in the first 6 hours, with metabolites increasing at later times. The data are shown in figure 2. Plasma half-times for doses of 1000–2000 mg/m² were 60–120 minutes. When daily doses × 5 days were given, the 24-hour excretions still approximated 80% on subsequent days. The balance of the dose may be disposed of by other means not monitored (eg, perspiration and feces). Plasma levels in the patients given repeated daily doses were routinely monitored at an early time (30 minutes) and just prior to the next dose to determine maximum and minimum levels achieved. These analyses are summarized in table 1. In general there was a relationship between plasma levels and dose at doses < 1500 mg/m², but increases in dose above this level had less evident effects.

Twenty-four-hour urine samples from five patients receiving single doses of ^{35}S-α-TGdR were examined by chromatographic separations. In two instances there was a major metabolite (or metabolites) amounting to approximately 85% of the radioactivity not present as ^{35}S-α-TGdR. This material remained at the origin on all three paper chromatography development systems and could not be eluted from Dowex-1 formate, cellulose, or charcoal columns. In the other three urine samples examined, one minor and two major metabolites were found. One patient (No. 2) with chronic myelogenous leukemia is representative of these values in terms of the metabolites found. The patient received 855 mg of ^{35}S-α-TGdR (450 mg/m²). In 24 hours, 55% of the dose was excreted, 17.5% as α-TGdR and 37% as metabolites. Since it contained mainly metabolites and little α-TGdR, the 10–24-hour urine sample was used for characterization. Aliquots of this urine sample were chromatographed directly on 3MM paper with the three development solvents. Also, a large aliquot (20 ml) was passed through a column of 1-g acid-washed Norit A charcoal:celite (50:50 by weight); the column was washed with water and eluted with 15 ml of 95% ethanol:water:15 M NH_4OH (70:24:6 by volume). An 81% recovery of metabolites with little change in ratio was achieved, and this de-salted eluate was used to compare on

chromatograms with direct urine aliquots. Such chromatograms gave clearer but similar results. Chromatograms were run off the papers to obtain adequate separations in some cases. Results with solvent systems (a), (b), and (c) are presented in table 2. Metabolites A, B, and C contained 7.5%, 36.0%, and 56.0% of the metabolite radioactivity, respectively. All three yielded 6-TG when submitted to acid hydrolysis (0.5 M HCl, 5 minutes at 90°C). No radioactivity was found in sulfate when barium precipitations were carried out on the urine with or without acid hydrolysis. Incubation of the metabolites with β-glucuronidase did not change chromatographic properties.

Further dose escalation to > 4000 mg/m²/day did not seem indicated. Blood levels attained with this and lower doses were above those found necessary to kill human lymphoblastic cells (WI-L2 cells, unpublished data) in culture, cells that possessed a moderate capacity to phosphorylate α-TGdR.

Table 2.—Chromatographic mobilities on 3MM paper chromatograms*

Compounds	Development solvent		
	(a)	(b)	(c)
6-TU	0.24	0.22	0.11
6-TX	0.24	0.38	0.37
6-TG	0.25	0.43	0.46
6-Methylthioguanine	0.27	0.65	0.62
α-TGdR	0.50	0.55	0.49
Metabolite A	0.52	0.47	0.28
Metabolite B	0.49	0.65	0.54
Metabolite C	0.47	0.43	0.67

*Aliquots of urine or "charcoal-purified" (*see* text) metabolite fraction were spotted on Whatman 3MM paper sheets and developed by descending chromatography with the solvents to the equivalent of a 600-mm distance. 5-mm strips were cut out and counted for radioactivity from each sample path. Mercaptoethanol (0.01 M) was added to the solvent systems. R_f values for metabolites on system (a) had to be conducted with material recycled from systems (b) and (c).

DISCUSSION

α-TGdR appears to be readily distributed in body water and relatively rapidly excreted, largely in the urine. There do not appear to be any unacceptable toxic effects. Both the lack of bone marrow suppression and the seeming absence of 6-TG or its usual metabolites (6-TX and 6-TU) would indicate that human tissues do not appreciably convert α-TGdR to 6-TG, which is perhaps more important than the nature of the catabolism of α-TGdR.

REFERENCES

1. IWAMOTO RH, ACTON EM, and GOODMAN L. 2′-Deoxythioguanosine and related nucleosides. J Med Chem 6:684–688, 1963.
2. LEPAGE GA, JUNGA IG, and BOWMAN B. Biochemical and carcinostatic effects of 2′-deoxythioguanosine. Cancer Res 24:835–840, 1964.
3. LEPAGE GA. The metabolism of α-2′-deoxythioguanosine in murine tumor cells. Can J Biochem 46:655–666, 1968.
4. PEERY A, and LEPAGE GA. Nucleotide formation from α- and β-deoxythioguanosine in extracts of murine and human tissues. Cancer Res 29:617–623, 1969.
5. NAKAI Y, and LEPAGE GA. Characterization of the kinase(s) involved in phosphorylation of α- and β-deoxythioguanosine. Cancer Res 32:2445–2451, 1972.
6. TAMAOKI T, and LEPAGE GA. Inhibition of DNA chain growth by α-2′-deoxythioguanosine. Cancer Res 35:1015–1017, 1975.
7. HENRY MC, and DIDOMENICO E. Preclinical toxicology of α-2′-deoxythioguanosine (NSC-71851). Cancer Chemother Rep Part 3, vol 5(1):9–14, 1974.
8. BRAY GA. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal Biochem 1:279–285, 1960.

Pharmacokinetic Study of *cis*-Dichlorodiammineplatinum(II) (DDP) in the Beagle Dog: Thermodynamic and Kinetic Behavior of DDP in a Biologic Milieu [1]

Andre F. LeRoy, Robert J. Lutz, Robert L. Dedrick,* Charles L. Litterst, and Anthony M. Guarino [2,3]

SUMMARY

This paper reports the development of a flow-limited pharmacokinetic model for *cis*-dichlorodiammineplatinum(II) (DDP) based on in vivo distribution of platinum (Pt) in organs, tissues, and body fluids followed up to 12 days in normal adult female beagle dogs treated with a single iv dose of 1 mg/kg. The pharmacokinetic model incorporated the following parameters which contribute to the overall disposition of the Pt compounds: (*a*) first-order rate constant for the disappearance of parent DDP ($k_m = 0.00416$ minute^{-1}); (*b*) kidney clearance of parent DDP ($k_{K-A} = 50$ ml/minute); (*c*) kidney clearance of metabolized Pt species ($k_{K-B} = 0.6$ ml/minute); and (*d*) biliary clearance of metabolized Pt species ($k_{B-B} = 0.035$ ml/minute). Flameless atomic absorption spectrophotometry was used to measure the Pt content of the biologic samples. The concentration of Pt increased sharply after administration of the drug in all body fluids and tissues examined except for the brain. This initial concentration rise was followed by several hours of very rapid decline and then by a period of much slower decline extending over the 12 days. On the basis of observed behavior of DDP in vitro and in vivo, we have inferred that the drug is transformed to a metabolite (or metabolites which can be represented by a single species) by a process, DDP $\rightleftharpoons$ metabolite(s), where the equilibrium is far to the right. This accounts for the two types of behavior of Pt-containing chemical species in the body: one moiety is rapidly cleared (half-life ~ 2 hours), and the second is much more slowly cleared (half-life ~ days). Results of our in vitro binding experiments support such a model. They show that some converted form of the parent Pt compound binds to constituents of dog plasma so that ultrafiltrates contain progressively less Pt over a 48-hour period. Enzymatic processes are ruled out because similar results are obtained when the Pt complex is incubated with bovine serum albumin in physiologic saline but not in normal saline alone. A scheme of reactions of Pt complexes is presented. Thermodynamic and kinetic data on DDP and its complex hydrolysis products are given as a basis for more fundamental understanding of the transformations that DDP may undergo. In addition, a thermodynamic model describing the expected behavior of these complexes in aqueous media with ionic compositions (Cl^- and pH) similar to those of the biologic milieu is discussed.

[Cancer Treat Rep 63:59/71, 1979]

---

[1]Received June 19, 1978; revised Nov 7, 1978; accepted Nov 13, 1978.

[2]Biomedical Engineering and Instrumentation Branch, Division of Research Services (*A. F. LeRoy, R. J. Lutz,* and *R. L. Dedrick*), and Laboratory of Toxicology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (*C. L. Litterst* and *A. M. Guarino*), National Institutes of Health, Bethesda, Md.

[3]We wish to express our appreciation for the excellent technical assistance of Peter Golueke, Edward Mimnaugh, Harry L. Sponseller, and Margaret Wehling in performing some of the experiments reported in this work, for the thorough review of this material and the numerous helpful suggestions of Dr. Jerry Collins, and for the excellent secretarial assistance of Linda Reams, Teresa Troy, and Annette Barb in the preparation of this manuscript.

**Reprint requests to:* Dr. Robert L. Dedrick, Biomedical Engineering and Instrumentation Branch, Division of Research Services, Bldg 13, Rm 3W13, National Institutes of Health, Bethesda, Md 20014.

The behavior of *cis*-dichlorodiammineplatinum(II) (DDP) as an antineoplastic agent has been studied in many animal experiments and in phase I and II clinical trials. It has been found to be effective both alone and in combination therapy for several types of tumors, and clinicians have developed techniques of administering this drug that reduce its renal toxicity and other untoward effects to tolerable levels. There has been an interest in improving the understanding of its mechanism of action.

To elucidate the behavior of DDP, we have conducted extensive measurements of platinum (Pt) distribution in the beagle as a function of time. On the basis of these measurements and studies of Pt binding to components of blood plasma, we have developed and described a flow-limited pharmacokinetic model for DDP in the beagle.

Chemical kinetic data together with a set of equilibrium relationships for the complex species offer a model which may help in gaining insight into the chemical speciation of the Pt in a relatively simple, protein-free environment. These data are presented in the Appendix for ready reference. Some of the implications of in vitro DDP behavior in the biologic milieu are developed more fully in the Appendix also.

MATERIALS AND METHODS

This work required development of the experimental procedure used in quantitation of Pt in tissue and body fluid samples, in vitro studies of chemical binding of Pt species by use of ultrafiltration, and studies of Pt distribution in dogs over time intervals ranging from 5 minutes to 12 days following single-dose iv administration of DDP.

The DDP used in this work was supplied by the Drug Development Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). Batch No. 10227-5A meets a Minimum Acceptable Purity specification of 98% with < 1% Magnus salt; other physicochemical characteristics such as solubility, elemental composition, infra-red spectrum, ultraviolet absorption, maximum molar absorptivity, and behavior on paper chromatography of the Pt complex are set forth in a summary of chemical information by C. A. Hewitt (Drug Development Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, May 18, 1971) that was supplied with the compound.

Platinum quantitation.—Details of the Pt assay and its validity are described elsewhere (1). The method can be used to detect Pt concentrations down to ~30 parts-per-billion in the biologic samples studied. Briefly, analyses of Pt in tissue and body fluids were made by use of a Perkin-Elmer Model 303 atomic absorption spectrophotometer equipped with a heated graphite atomizer (HGA-2000); the temperature control was modified with a slewing circuit designed and built in the Biomedical Engineering and Instrumentation Branch to produce a linear increase in temperature with time (1). Tissue samples were digested, first with nitric acid and then with a mixture of nitric and perchloric acids. After digestion and evaporation to near dryness, the residue was taken up in dilute hydrochloric acid. A small aliquot (20–30 μl) of this hydrochloric acid solution was then analyzed for Pt in the graphite furnace. Plasma and urine samples were assayed without prior digestion; each was compared with appropriate standards to take matrix effects into account. Validity of the Pt assay technique in various tissues and fluids was confirmed by neutron activation analysis. The standard error of the mean for typical analyses was estimated to be 5%–8% by flameless atomic absorption spectrophotometry. Both methods yield total Pt content, ie, all chemical species containing Pt; no attempt was made to determine the chemical forms of Pt in the various tissues and fluids analyzed in this experimental work on the beagle.

In vitro binding—ultrafiltration studies.—DDP was incubated at 37° C in the dark at concentrations of 1 and 5 μg/ml for up to 48 hours in the following media: (*a*) 0.85% wt/vol NaCl (normal saline [NS]); (*b*) 0.1 mM bovine serum albumin (BSA) in NS; and (*c*) heparinized canine plasma. Triplicate 3.0-ml samples were withdrawn, and 0.5 ml of each was set aside for analysis. The remaining 2.5 ml was placed into Centriflo Ultrafiltration cones (Amicon 2100, CF-50) and centrifuged for 30 minutes (NS and BSA for 15 minutes) at 200 × *g* in a refrigerated (0°C–4°C) International Model PR-2 centrifuge. The Pt recovered in the ultrafiltrate was taken as the unbound fraction.

In vivo studies.—Adult female beagle dogs (8–10 kg) were anesthetized with iv (cephalic vein) pentobarbital sodium (30 mg/kg). A catheter was inserted into the external jugular vein for the collection of blood samples, and a human pediatric urinary catheter was passed through the urethra into the urinary bladder for the collection of urine. DDP was administered iv (1 mg/kg in 0.85% wt/vol NaCl solution) through an indwelling catheter in the femoral vein.

Fluid sampling.—Blood and urine samples were taken for Pt analysis starting 5 minutes after drug injection. Catheters remained in place up to 4 hours. Then the dogs were either killed by exsanguination (ie, while still under anesthesia) or the catheters were removed, the wounds were closed and sutured,

and the dogs were returned to their cages. Food was withheld for 16 hours after drug administration. For studies lasting > 4 hours, blood was sampled from the cephalic vein. Urine and urinary salt residues left by evaporation were collected from the cage pans. Bile was also analyzed.

Tissue sampling.—The tissue samples studied were taken from dogs killed at 10, 30, and 60 minutes, 4 and 24 hours, and 4, 6, and 12 days after drug administration. Data reported for 10 and 60 minutes and 12 days represented tissues from two dogs each, while data for 4 days represented three dogs. Dogs studied for 1, 4, 6, and 12 days were killed by electrocution. Samples of tissues analyzed for Pt were lung, liver, kidney, uterus, ovary, skeletal muscle, skin, adrenal, spleen, fat, pancreas, heart, stomach, small intestine, brain, and ascending colon. Blood plasma and urine samples were collected frequently throughout the time interval. As a result of sequential blood sampling in each dog, several data points for Pt blood concentration were available at many of the specified time points. The tissue samples at times > 4 days were kidney, liver, lung, skin, muscle, and ovary. Bile samples were routinely taken from the gallbladder when animals were killed. Blood samples were analyzed for blood urea nitrogen and creatinine to evaluate renal function for those dogs studied for > 24 hours. The tissue samples analyzed were those from one dog at each time point except in those instances where tissue data were available from more than one dog and then the average value was used. Three to six plasma samples and two or three urine samples were collected from different dogs at each time point.

DEVELOPMENT OF A PHARMACOKINETIC MODEL

A physiologic pharmacokinetic model for the distribution, disposition, and excretion of DDP and its major metabolites in the dog is illustrated by the flow diagram in figure 1. The system is composed of several compartments which represent the primary organs or anatomic body regions where Pt is found. The rate of drug transport to the vascular space of these compartments is determined by their individual blood perfusion rates, illustrated by the arrows in figure 1. For this particular model, the following assumptions were made:

DDP and its metabolites in the arterial blood equilibrated rapidly with the tissue space of each compartment so that the tissue concentration was in equilibrium with the venous blood concentration in each compartment. Such a model is referred to as a "flow-limited" model and implies that the transport of free drug across tissue cell membranes is relatively fast compared with the tissue blood perfusion rate. While protein-bound Pt species may not cross the cell membrane themselves, they are presumed to be in equilibrium with unbound Pt complexes that do. As the unbound forms cross into cells, the bound forms in the extracellular space shift rapidly to the unbound forms to restore the equilibrium. Therefore, protein binding of any of the drug species does not preclude use of a flow-limited model. As an example, this has been shown to be valid for thiopental for which the equilibrium is far in the direction of bound drug (2).

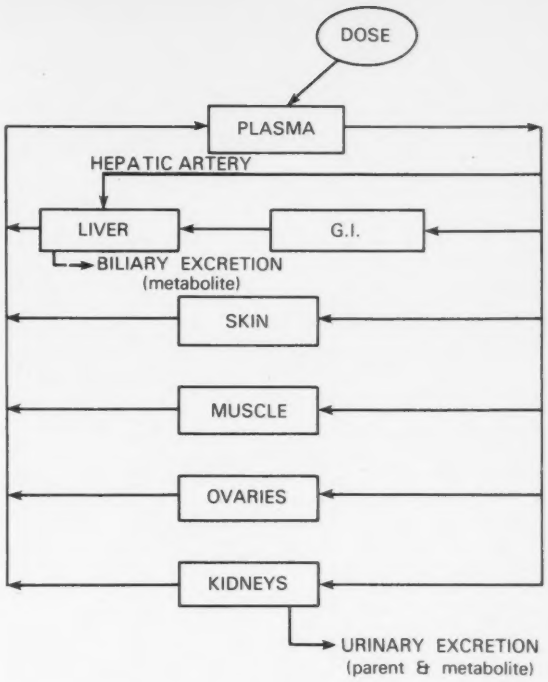


FIGURE 1.—Diagram of flow-limited pharmacokinetic model of DDP in the dog.

Metabolism of DDP occurred in the aqueous space of all compartments of the model. This assumption was based on data which indicated that DDP was reactive in nonbiologic aqueous media. Details of the reaction scheme used in the pharmacokinetic model along with its attendant assumptions are given below.

Elimination of the Pt from the animal occurred by both the urinary and biliary excretion routes. In the model, we assumed that the kidney eliminated both DDP and its metabolites but that the liver eliminated only metabolites.

The physiologic pharmacokinetic model, then, is a

TABLE 1.—Pharmacokinetic parameters for DDP in a 10-kg dog*

Compartment	Volume, V† (ml)	Plasma flow rate, Q† (ml/min)	Pt distribution coefficient, R‡
Plasma	410	564.5	—
Muscle	4600	115	0.7
Skin	1600	66 (ref 7)	3.5
Ovaries	2 (ref 8)	0.5 (ref 8)	1
Liver	400	240 (ref 9)	6
Kidneys	50	75	8
Gastrointestinal	400	68	1

*Kidney clearance of parent, k_{K-A} = .50 ml/min; kidney clearance of metabolite, k_{K-B} = 0.6 ml/min; metabolic rate constant, k_m = 0.00416 min^{-1}; and biliary clearance of metabolite, k_B = 0.035 ml/min.

†Volumes and flow rates obtained from ref 6, scaled to a 10-kg dog except when noted otherwise.

‡Distribution coefficient, R, for parent DDP and metabolite assumed equal.

series of differential equations representing mass balances on each of the compartments in the model. Once the necessary model parameters were determined, such as blood flow rates, tissue volumes, distribution coefficients, biliary and urinary clearance rates, and metabolism constants, the set of equations was solved numerically for a particular dose magnitude and schedule. The resultant solution gave the predicted parent and metabolite concentrations in each tissue and the amount of drug excreted in the urine and bile. More detailed discussions of physiologic pharmacokinetic models have been presented in the literature (3–5).

Determination of Model Parameters

The following section describes the methods and assumptions utilized to determine the required model parameters which are listed in table 1.

Organ volumes, V, and perfusion rates, Q.—Estimates of organ volumes and blood (or plasma) perfusion rates for a standard beagle dog were obtained from the literature. Appropriate references are cited in table 1.

Distribution coefficient, R.—The equilibrium distribution coefficient, R, for each compartment was determined from the experimental data by taking the ratio of the Pt concentration in the tissue to the Pt concentration in the plasma. At sufficiently long times (in this case, > 48 hours), the tissue and plasma have reached equilibrium and most of the Pt is in the form of metabolite; thus R at this point represents the distribution rates for metabolite. Since the tissue-to-plasma distribution ratios for the parent DDP calculated for similar tissues at early times (10–60 minutes) are similar to those for one metabolite at the longer times, the values for R reported in table 1 apply to both parent and metabolite.

Kidney clearance, k_K.—Kidney clearance was estimated by plotting the cumulative amount of Pt in the urine against the area under the curve of Pt concentration in plasma versus time, and taking the slope of this line. The calculations for the clearance of DDP were restricted to relatively short times (between 5 and 20 minutes) to ensure that the Pt concentration was primarily that of the parent compound. Kidney clearance of the metabolite was estimated in a similar manner from urinary excretion data at longer times when Pt in the urine and in the plasma was assumed to be dominated by metabolite.

Biliary clearance, k_B.—Biliary clearance of metabolite was estimated from the rate of excretion of Pt into gallbladder bile. The rate of excretion was calculated by multiplying the measured concentration of Pt in the bile by an estimate of the biliary flow rate for a 10-kg beagle dog (10), and the product was equated to the biliary clearance k_B multiplied by the Pt plasma concentration. The data chosen for these calculations were for times of ≥ 4 days when the plasma and bile concentrations were relatively constant and most of the measured Pt would be expected to be metabolite. The biliary clearance of the DDP was neglected, since little Pt appeared in short-term bile when most of the Pt would be expected to be parent.

Metabolism constant, k_m.—Some of the reactions of DDP in aqueous as well as in biologic media are illustrated in figure 2. *cis*-$[Pt(NH_3)_2Cl_2]$ undergoes seriatim substitution of aquo ligands for both chloro ligands; the reactions are reversible and kinetically slow (half-life ~ 6–8 hours at 25°C). The deprotonation (protonation) reactions of the substituted aquo (hydroxo) ligands are reckoned as nearly instantaneous (half-life ~ milliseconds) in such complexes. The successive aquation steps replacing chloro ligands are reversible, and the equilibrium position of these reactions depends on the initial DDP concentration as well as the chloride concentration of the reaction medium; thus, the relative amounts of the several chemical species may shift dramatically (though perhaps slowly) with changes in chloride concentration. These transformations are shown in figure 2. In this reaction scheme, the ligand exchange reactions are written as proceeding formally by an aquation step in the forward direction before deprotonation of an aquo ligand may take place. The rates and mechanisms of these reactions are discussed further in the Appendix. It is evident that this reaction scheme is not simple, and experimental

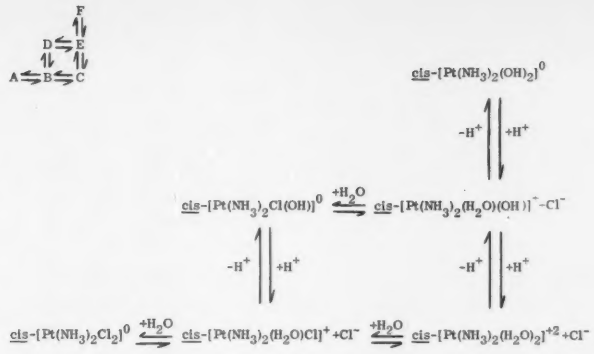


FIGURE 2.—Aquation-anation and protonation-deprotonation reactions of *cis*-$[Pt(NH_3)_2Cl_2]$. Note that species A, D, and F are uncharged. Inset: Letter designation of *cis*-Pt ammine species with chloro, aquo, and hydroxo ligands.

measurement of the concentration of the many complex species is a formidable task. Since these data are not available to us, and in order to make the pharmacokinetic modeling tractable, the in vivo reaction scheme was simplified in the model to assume that the parent compound was metabolized in all tissues by a first-order reaction, and that the many possible metabolites could be represented kinetically by a single species. This simplification is probably a good approximation to the actual system since many complex reaction schemes have a single rate-controlling step. The first-order reaction constant, k_m, was calculated for the model from in vitro ultrafiltration data in which parent compound was incubated with dog serum. Since the parent compound was nearly 100% filterable from serum and the incubation product was not, parent depletion and therefore product formation were followed with time by measuring the filterable fraction of Pt in the dog serum (*see* fig 3).

RESULTS AND DISCUSSION

A precise pharmacokinetic model for the distribution and disposition of DDP is complicated by the diversity of reactions that the parent compound can undergo in vivo. The complexity of the reaction scheme for DDP in a simple in vitro aqueous environment has already been described (11) and is illustrated in figure 2. It would be difficult or impossible to determine the various parameters required by the model for each metabolite, for example, the distribution coefficients, the kidney and biliary clearance values, and the metabolism rates. To overcome the difficulty would require measurement of the concentration of each Pt species as a function of time in all tissues and excreta. Approximations can be made which at least allow us to describe the apparent rate of disappearance of the parent DDP by reaction and its eventual distribution into and excretion from the body. The ultrafiltration study provided an in vitro method for measuring the rate of transformation of DDP to its reaction products.

These experiments yielded the results plotted in figure 3. They show that the filterable fraction of Pt in NS remains substantially unchanged during the 48-hour interval studied, while in plasma it diminishes as shown.

Incubation of DDP with canine plasma at 37°C for 48 hours left 5%–8% of the Pt filterable through a 50,000 MW cutoff ultrafilter. The rate of diminution is independent of drug concentration for the two values studied, which supports the assumed first-order reaction mechanism. Samples taken for Pt analysis shortly after the start of incubation (0+) and at 3 and 48 hours for the DDP in BSA-NS showed behavior similar to that for plasma.

We interpret these findings to mean that parent DDP is not appreciably bound to serum protein and that the binding of the Pt compounds occurs after an aquation step. This conversion by nonenzymatic processes results in one or more products that are at least 92%–95% bound. It is unlikely that any uncomplexed Pt ions result from the reactions in our in vitro set up. The stability of the Pt-NH_3 bond is so great that the Pt complex bound to protein almost certainly retains the two ammine groups in the *cis* positions in the original DDP. On the basis of this information, the reaction scheme was simplified assuming that

$$\text{DDP} \xrightarrow{k_m} \text{[bound products]}$$

and that the rate of parent depletion and therefore product formation could be followed with time by

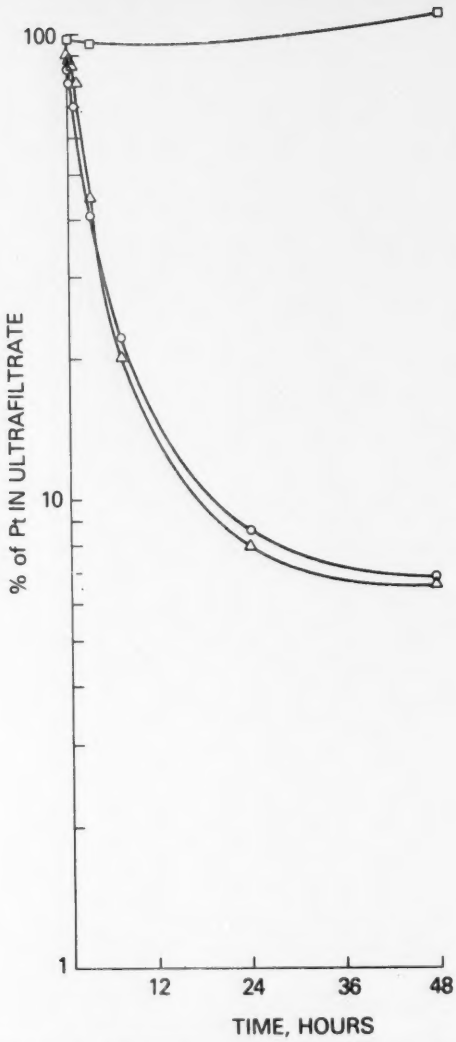


FIGURE 3.—% of Pt in ultrafiltrate as a function of time of incubation. △ = $[DDP]_0$ (5 μg/ml of plasma); ○ = $[DDP]_0$ (1 μg/ml of plasma); and □ = $[DDP]_0$ (5 μg/ml of NS).

measuring the filterable fraction. The first-order rate constant was determined from the initial slope (near t = 0) in figure 3 to be 0.00416 $minute^{-1}$. This number compares well with the rate constant for hydrolysis of DDP in aqueous media of 0.006 $minute^{-1}$ (Appendix). Apparently, the equilibrium binding of the reaction products was rapid compared with the rate-limiting reaction (aquation) step.

In addition to the reaction rates, information is required for the model which describes the excretion of the various Pt species (parent and metabolites). The primary excretion is by the kidney.

Pt appears in urine very shortly after iv administration of the drug. Renal clearance of Pt complexes is rapid initially, then falls off to a much lower rate. Cumulative urinary Pt excretion rose rapidly with almost 60% of the administered dose collected within the 4 hours after treatment. Since the dogs were catheterized during this time, urine collection and thus urinary Pt excretion were taken to be quantitative. Figure 4 shows the cumulative amount of Pt in the urine of one dog versus the integral under the plasma concentration curve for times up to 4 hours. After a brief delay, presumably associated with flow of the filtered Pt from the glomerulus to the bladder, there is a rapid rise in Pt excreted. For constant clearance of a drug, figure 4 would be a straight line with a slope equal to the kidney clearance of that drug. Figure 4, however, is a curved line with a decreasing slope. The initial slope (after 5 minutes' delay and up to about 20 minutes) represents the initial clearance of Pt as parent DDP. This value is 50 ml/minute which is of the order of the glomerular filtration rate for a dog. The decreasing slope at later times implies a decreasing clearance of Pt. This can occur for at least two reasons. First, parent DDP can be converted to metabolites which begin to dominate in the plasma, and whose clearance values are lower than that of the parent DDP, causing the overall clearance of Pt to fall. Second, the metabolic products are most likely bound to plasma protein. Though this binding is not irreversible, it can effectively reduce the free concentration of Pt species in the plasma, causing an effective decrease in the clearance value of Pt as calculated by the method in figure 4, taking the slope at later times. The latter hypothesis seems more likely. In any event, the value of the second clearance (0.6 ml/minute) calculated for the model represents an effective clearance for products of the DDP reaction based on analysis of total Pt (free or bound), and this number provided reasonable simulation of the long half-life disappearance of Pt illustrated by the prediction in figure 5.

Only small quantities of Pt were detected in the bile, and these were observed only at times sufficiently long to assume that they represent Pt metabolites. Biliary secretion was not a significant route of elimination as evidenced by the relatively small value of biliary clearance, 0.035 ml/minute, compared with kidney clearance (0.6 ml/minute).

The results obtained with the pharmacokinetic model are shown in figures 5, 6, and 7; the experimental values for the tissues kidney, liver, skin, plasma, and muscle are shown as discrete points while the solid lines are the values obtained from the mathematic model. Experimental values obtained for the plasma are shown in two separate

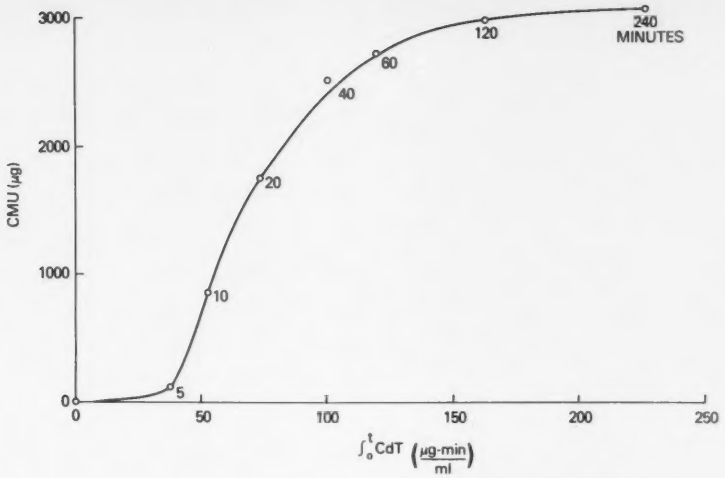


FIGURE 4.—Cumulative amount of Pt in urine vs integral under the plasma concentration curve to 4 hrs.

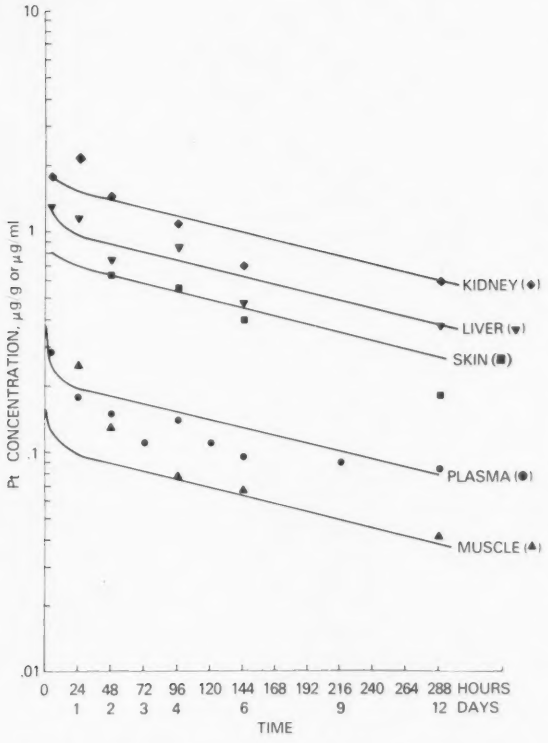


FIGURE 5.—Pt concentration as a function of time for 12 days after a single iv dose of 1 mg of DDP/kg in the dog. Solid curves represent model simulations.

figures (6 and 7) to reflect the details of the short-term (0–4 hours) and long-term (up to 12 days) studies. Table 2 lists the experimentally determined Pt concentration in numerous other organs not plotted in the figures. A partial presentation of these data appeared earlier in a preliminary report (12) with minimal analysis. Further, plasma Pt concentrations reported here have been corrected for a strong matrix effect. The basis for correction had not been developed when the earlier report was published.

The most serious discrepancy between the model simulations and the data occurs for the muscle compartment. At times up to 1 hour, the model overpredicts the muscle concentration, whereas from 4 to 24 hours, it underpredicts the concentration. This might indicate a departure from the flow-limited assumption for the muscle compartment. The data show a behavior which might be expected if the muscle tissue had a restricted permeability to the Pt species present. In such a case, Pt would enter the cells more slowly, but also would be retained for longer periods of time than the model predicts. When data on the relative concentrations of parent DDP and its metabolites in tissue and plasma become available, the model can be improved to account for membrane transport limitations to the various Pt species.

Figures 6 and 7 show the computed plasma concentrations for the parent compound (DDP), metabolite, and total Pt (parent + metabolite) for the time intervals of 0–4 and 0–24 hours. These figures show that the model allows us to predict the relative

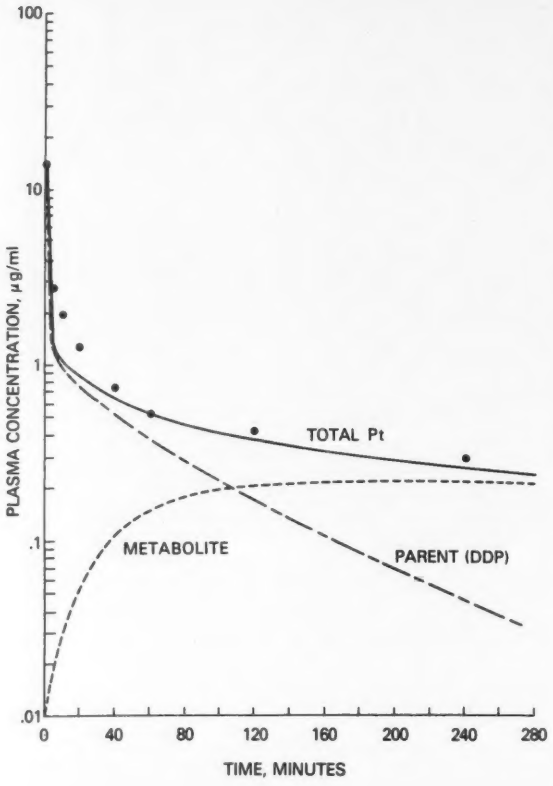


FIGURE 6.—Computed plasma concentration for DDP, metabolite, and total Pt for the time interval 0–4 hrs. Experimental data points for total Pt are indicated by the solid circles and represent averages of values from 3–5 dogs at each time point.

concentrations of DDP and its cumulative metabolic products containing Pt in plasma. Similar predictions can be made for the other tissues in the model. In figure 6, the metabolite concentration is seen to rise gradually with time so that by 2 hours, the parent and metabolite concentrations are equal in the plasma. The simulations in figure 7 indicate that by 8 hours the Pt in the plasma is primarily in the form of metabolites, and that the DDP continues to diminish in concentration as it is eliminated from the body.

CONCLUSIONS

A physiologic pharmacokinetic model was derived for DDP based primarily on parameters determined from physiologic and anatomic data in the literature or from independent in vitro experiments.

After an appropriate simplifying approximation to the complex reaction scheme for DDP, the model allows for prediction of the concentration in plasma, tissues, and excreta of both parent DDP and its primary metabolite(s), the latter being difficult to measure experimentally.

DDP does not bind appreciably to protein; it undergoes transformation nonenzymatically in aqueous media to a series of metabolites that do bind strongly to BSA or other plasma protein. This transformation appears to be first-order and can be followed in vitro by an ultrafiltration experiment. The in vitro rate constant of 0.00416 minute^{-1} was applied in the model also as the in vivo metabolism rate in the aqueous regions of each model compartment. The rate-limiting step is probably the first aquation step, and our rate constant compares favorably with the value of 0.006 minute^{-1} for the

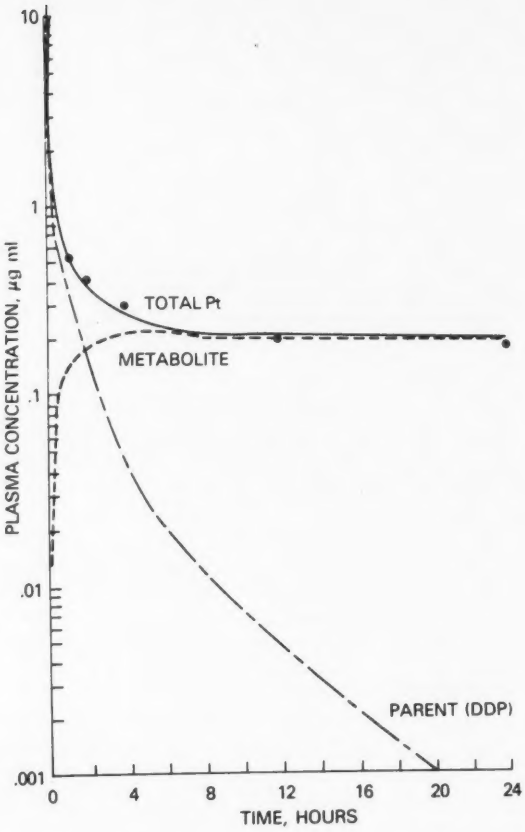


FIGURE 7.—Computed plasma concentration for DDP, metabolite, and total Pt for the time interval 0–24 hrs. Experimental data points for total Pt are indicated by the solid circle.

TABLE 2.—Organ distribution of Pt following iv administration of 1 mg/kg of DDP to female beagle dogs*

	Time								
	Mins			Hrs		Days			
Organ	10	30	60	4	24	2	4†	6	12
Plasma	2.08	0.38	0.54	0.26	0.20	0.16	0.15	0.10	0.10
Kidney cortex	5.41	3.07	1.75	1.75	2.18	1.58	1.21	0.86	0.78
Kidney medulla	7.88	2.85	2.15	1.99	2.11	1.16	0.60	0.36	0.41
Ovary	1.81	1.18	0.64	1.04	0.78	—	0.41	—	—
Uterus	—	—	—	—	—	—	0.85	—	—
Uterus horn	1.37	0.85	0.77	1.35	—	—	—	—	—
Uterus body	1.35	1.35	0.62	1.01	—	—	—	—	—
Liver	—	—	—	1.34	1.17	—	0.93	—	—
Liver tip	1.64	1.70	1.27	—	—	—	—	—	—
Liver body	1.37	1.38	1.51	—	—	0.75	—	0.48	0.39
Skin	1.25	0.60	0.16	—	—	0.61	0.54	0.40	0.18
Lung	—	—	—	—	0.80	—	0.37	—	—
Lung tip	1.06	1.16	0.68	0.71	—	—	—	—	—
Lung body	1.36	1.25	0.92	0.86	—	—	0.47	—	0.22
Stomach	1.20	—	0.68	—	—	—	0.17	—	—
Adrenals	1.06	—	0.34	0.93	1.08	—	0.14	—	—
Colon	0.70	—	0.40	—	—	—	0.13	—	—
Muscle	0.26	0.20	0.24	0.24	0.25	0.13	0.08	0.06	0.07
Heart atrium	—	—	—	0.44	0.26	—	0.16	—	—
Heart ventricle	0.32	—	0.11	0.22	—	—	0.05	—	—
Small intestine	0.31	—	0.35	—	—	—	0.22	—	—
Pancreas	0.28	—	0.18	0.47	0.66	—	0.31	—	—
Spleen	0.26	—	0.15	0.52	0.43	—	0.35	—	—
Brain	—	—	—	—	< 0.06	—	0.008	—	—
Fat	1.63	—	0.24	0.23	< 0.04	—	0.04	—	—
Bile	0.03	—	0.24	0.51	3.08	0.36	0.04	0.06	—

*All values are stated in μg of Pt/g (wet weight) or μg/ml.
†Day 4 values represent data from 3 dogs. Values from the 1 dog receiving 1.8 mg/kg of DDP have been normalized to 1 mg/kg/dose.

hydrolysis of DDP calculated from data presented in the literature.

The primary mode of excretion of DDP and its metabolites is by the kidney. The excretion occurs in two phases, first a rapid clearance of parent DDP (clearance = 50 ml/minute) and second a slower clearance of metabolites (clearance = 0.6 ml/minute). This later value is an effective clearance based on measurement of total Pt in the plasma and probably reflects the binding of the Pt metabolites to serum proteins, thereby reducing the actual free concentration in the plasma. When utilized in the flow-limited model, this second clearance of 0.6 ml/minute simulates the long half-life of Pt out to 12 days quite well.

Biliary secretion of Pt is quite small compared with kidney clearance and appears to occur primarily for the metabolite with a clearance of 0.035 ml/minute.

The flow-limited model provided a reasonable prediction of most of the tissue data. Lack of specific analysis of the metabolic forms of the Pt at this time precludes a more detailed analysis of the transport. The muscle compartment provided the most serious discrepancy between simulations and data and may reflect a mode of transport other than flow-limited, for example, low cell membrane permeability.

APPENDIX

THERMODYNAMICS: KINETICS AND MECHANISM OF DDP REACTIONS IN AQUEOUS MEDIA

Mechanisms of Ligand-Exchange Reactions in Chloro-ammine Pt(II) Complexes

DDP may be represented as a four-coordinate complex in which the central metal atom and the four attached donor atoms lie approximately in the same plane. Such square-planar complexes are largely found among the states of the low-spin d^8 system; they have dsp^2 hybridization.

Substitution reactions of Pt(II) complexes proceed with retention of geometric configuration; that is, *cis* complexes give *cis* products and *trans* complexes give *trans* products. Substitution reactions of several different Pt(II) complexes with a variety of reagents are bimolecular and nucleophilic, designated $S_{N,2}$.

Studies of the $[Pt(NH_3)_2Cl_2]$ isomers have clearly established that the chlorides are the reactive ligands (13,14). Though NH_3 is low in the *trans*-effect series, the Pt-NH_3 bond is very stable (15).

Studies of the isotopic exchange of chloride ions and chloro ligands of the set of square-planar chloro-ammine complexes of Pt(II) show that it occurs by processes in which the chloro ligand is replaced in a reversible aquation or by the direct nucleophilic attack of chloride upon the complex (16–22). These exchange processes are first-order in the complex.

Martin and his students (20) have supplied considerable experimental evidence for an associative solvent path in demonstrating the lack of a large rate effect in the rates of hydrolyses for reactions of a number of Pt chloroammine complexes of differing net charge in aqueous medium. Their results suggest that in the transition state both bond making and bond breaking are important. In these reactions, the solvent path, like the direct displacement path, seems to be a bimolecular displacement process. Similarly, the rates of direct radiochloride ion exchange, k_{Cl}, with these Pt(II) complexes are only slightly affected by changes in the charge on the complex.

The similarity of reaction rates for Pt(II) complex species whose charges range from –2 to +1 indicates that a simple dissociation mechanism to a coordination number of 3 does not occur in the substitution reaction, but rather an associative path obtains. The results of Reishus and Martin (19) appear to support such a conclusion. It is likely that all the chloride exchange processes in the systems of $[PtCl_4]^{-2}$, $[Pt(NH_3)Cl_3]^-$, and *cis*-$[Pt(NH_3)_2Cl_2]$ occur by aquation (acid hydrolysis) and that the chloride ion cannot compete with the solvent in the replacement of a chloride ion under the concentration conditions studied. Apparently, the aquo ligand can be replaced rapidly by chloride or by many other substituting groups so that the rate of aquation controls the rate of many substitution reactions, including the rate of base hydrolysis.

Behavior of *cis*-$[Pt(NH_3)_2Cl_2]$ in Aqueous Solutions

A brief description of the transformations DDP undergoes in solution was given in the section entitled Development of a Pharmacokinetic Model. While DDP and the related tetrachloro and tetraammine of Pt(II) have been the subject of numerous studies, definitive thermodynamic and kinetic data on the transformations they undergo are incomplete. With the available information we have developed the following model.

Summary of Equilibrium Relationships

Based on the reaction scheme stated for DDP, the following equilibrium constant relationships may be written for the reaction scheme in figure 2. The equations are written to take account of the chloride concentration, $[Cl^-]_o$, of the medium (plasma, intracellular fluid, urine, cerebrospinal fluid, or other body fluid) and its pH; they do not take account of reactions of the complexes with other nucleophiles, eg, amino acids, nucleic acids, peptides, or proteins, reactions that may be important.

$$K_1 = \frac{[B][Cl^-]}{[A]} = \frac{[B]([Cl^-]_o + [B] + [D] + 2([C] + [E] + [F]))}{([A]_o - [B] - [C] - [D] - [E] - [F])} \quad [A\text{–}1]$$

$$K_2 = \frac{[C][Cl^-]}{[B]} = \frac{[C]([Cl^-]_o + [B] + [D] + 2([C] + [E] + [F]))}{[B]} \quad [A\text{–}2]$$

In the presence of bicarbonate (HCO^-_3) and/or phosphate ($H_2PO_4^-$ and HPO_4^{-2}) ions at millimolar concentration levels in body fluids and tissues, it can be assumed that the hydrogen concentration

remains near its initial value after drug administration at therapeutic levels (approximately 10^{-6} M), ie, $[H^+] = [H^+]_o$. The proton partial-equilibrium expressions for K_3, K_4, and K_5 can thus be expressed:

$$K_3 = \frac{[D][H^+]_o}{[B]} \qquad [A-3]$$

$$K_4 = \frac{[E][H^+]_o}{[C]} \qquad [A-4]$$

and

$$K_5 = \frac{[F][H^+]_o}{[E]}. \qquad [A-5]$$

Plasma is strongly buffered; only small changes in pH occur with resulting small effect on the equilibria of the protonated species. But since urinary pH can vary over a considerable range after drug treatment, pH data should be considered before using the simplified expressions [A–3], [A–4], and [A–5] for urine samples.

Values for Equilibrium Constants

Values of the equilibrium constants for the successive aquation reactions of A and B at 37°C are estimated from data at 25°C and 35°C recently reported by Lee and Martin (*see* table A–1).

Protonation-Deprotonation Equilibria

Aquo species such as B, C, and E in equation 4 may lose a proton to form the corresponding conjugate-base hydroxo species, D, E, and F. The pK_a values for the deprotonation reactions of C and E are given in table A–2. The pK_a for *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ was estimated to be 6.3 ± 0.1 as the basis of the relative pK_a values of analogous metal complexes and Brønsted acids under similar conditions. The pK_a values of these complexes have been taken to be constant over the temperature range considered.

The pK_a values for species C, B, and E used in the calculation of Pt distributions are stated in table A–2.

TABLE A-1.—Equilibrium constant data: for μ = 0.318 M adjusted with KNO_3

Temperature (C°)	K_1 (mol/liter)	K_2 (mol/liter)
25	$3.63 \pm 0.22 \times 10^{-3}$	$1.11 \pm 0.14 \times 10^{-4}$
35	$4.37 \pm 0.13 \times 10^{-3}$	$1.88 \pm 0.08 \times 10^{-4}$*
37	4.52×10^{-3}†	2.08×10^{-4}†
	$\Delta H^\circ_1 = 3.4 \pm 1.2$ kcal	
	$\Delta H^\circ_2 = 9.6 \pm 2$ kcal	

* A typographic error appears in ref 23 citing this value.
† Estimated using the Van't Hoff reaction isobar in the form:

$$-\ln K = \frac{\Delta H}{RT} + C$$

assuming ΔH = constant over the short range of temperatures considered.

TABLE A-2.—pK_a values for *cis*-$[Pt(NH_3)_2(H_2O)_2]^{+2}$ and related species

Species	Complex	pK_a at 20°C
C	*cis*-$[Pt(NH_3)_2(H_2O)_2]^{+2}$	5.6 (ref 16)
B	*cis*-$[Pt(NH_3)_2Cl(H_2O)]^{+1}$	6.3 (estimated)
E	*cis*-$[Pt(NH_3)_2(OH)(H_2O)]^{+1}$	7.3 (ref 24)

Calculated Pt Species Concentrations

The media examined in the in vivo and in vitro studies were buffered at values of pH sufficiently low to suggest that F would probably be present in negligible concentration over the pH range 5.0–7.5. With the use of the experimental values for K_1, K_2, K_4, and K_5, and the estimated K_3, for *cis*-$[Pt(NH_3)_2Cl(H_2O)]$ in the set of simultaneous equations [A–1]–[A–5], estimates were made of the equilibrium concentrations of each of the species A–F as a function of $[Cl^-]_o$ over a range of values covering that encountered in most mammalian species, and for pH values from 5.0 to 7.5 for $[A]_o = 10^{-6}$ M at 37°C. The results of these calculations are shown in figures A–1 and A–2. They indicate that an initial chloride ion concentration $[Cl^-]_o$ = 0.1 M and pH = 7.5 (approximate $[Cl^-]$ and pH of plasma, respectively) displaces the equilibria far to the left in figure 2, so that the A, B, and D species predominate [(A > 83 mol %), (B ~ 4 mol %), (D ~ 12 mol %)]; C, E, and F account for about 1 mol % total. At $[Cl^-]_o$ = 0.005 M (approximate intracellular $[Cl^-]$) the distribution shifts so that A is about 31 mol %, B is about 28 mol %, and D is about 32 mol %. E = ~ 7 mol % and C and F are about 1 mol % each.

At the low value of pH = 5 that may be found in the urine, the distribution of species is such that when $[Cl^-]$ = 0.1 M, A is > 95 mol % and B is > 4 mol % and when $[Cl^-]$ = 0.005 M, A is ~ 50 mol % and B is ~ 45 mol % while C and D each account for about 2 mol %.

Rate Constants for Hydrolysis of DDP

Rate constants for the first acid hydrolysis reaction step of *cis*-$[Pt(NH_3)_2Cl_2]$ have been measured at several temperatures by different workers (19,22,25).

The rate constant for acid hydrolysis of DDP at 37°C is estimated from Arrhenius plots of these data to be 10^{-4} sec^{-1} = k_1 (half-life = 1.9 hrs). This compares closely with the model metabolic rate constant, k_m, that was derived from the ultrafiltration experiment.

REFERENCES

1. LEROY AF, WEHLING ML, SPONSELLER HL, ET AL. Analysis of platinum in biological materials by flameless atomic absorption spectrophotometry. Biochem Med 18:184–191, 1977.
2. BISCHOFF KB, and DEDRICK RL. Thiopental pharmacokinetics. J Pharm Sci 57:1346–1351, 1968.
3. BISCHOFF KB, DEDRICK RL, and ZAHARKO DS. Preliminary model for methotrexate pharmacokinetics. J Pharm Sci 59:149–154, 1970.
4. BISCHOFF KB, DEDRICK RL, ZAHARKO DS, ET AL. Methotrexate pharmacokinetics. J Pharm Sci 60:1128–1133, 1971.
5. DEDRICK RL, ZAHARKO DS, and LUTZ RJ. Transport and binding of methotrexate in vivo. J Pharm Sci 62:882–890, 1973.
6. LUTZ RJ, GALBRAITH NM, DEDRICK RL, ET AL. A model for the kinetics of distribution of actinomycin-D in the beagle dog. J Pharmacol Exp Ther 200:469–478, 1977.
7. SAPERSTEIN LA. Regional blood flow by fractional dilution of indicators. Am J Physiol 193:161–168, 1958.
8. ANDERSON AC. The Beagle as an Experimental Dog. Ames, Iowa State University Press, 1970.
9. BURTON-OPITZ R. The vascularity of the liver. IV. The magnitude of the portal inflow. Q J Exp Physiol 41:113–125, 1911.

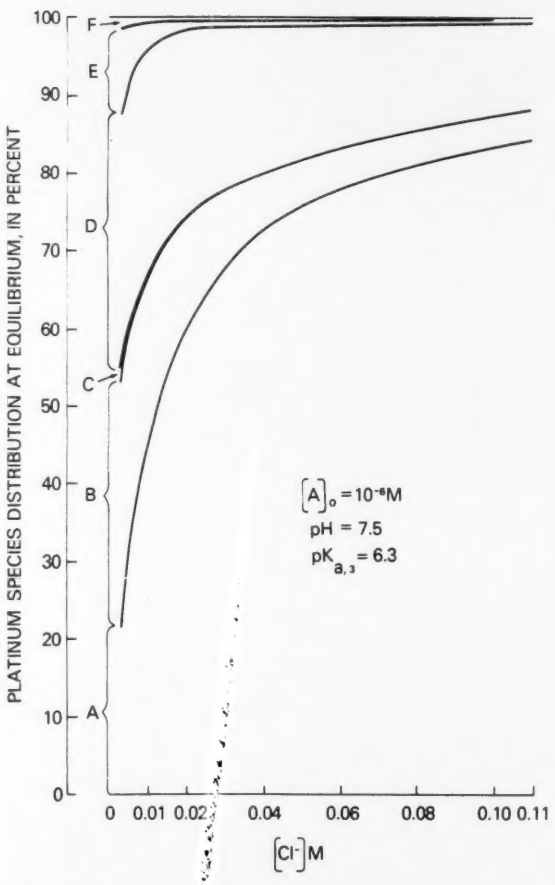


FIGURE A–1.—Computed Pt species distribution at equilibrium at 37°C and pH = 7.5 as a function of [Cl^-]. At each chloride concentration, the successively higher ordinates represent the cumulative sums of % distribution of Pt species A, B, C, D, E, and F. The proportion of each species is represented by the difference between ordinates. At all [Cl^-], the sum [A] + [B] + [C] + [D] + [E] + [F] = 100%.

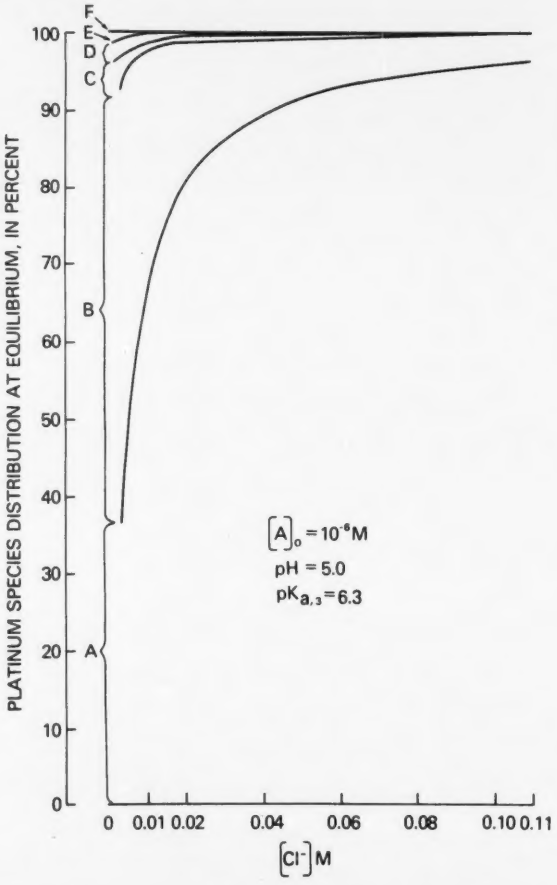


FIGURE A–2.—Computed Pt species distribution at equilibrium at 37°C and pH = 5.0 as a function of [Cl^-]. At each chloride concentration, the successively higher ordinates represent the cumulative sums of % distribution of Pt species A, B, C, D, E, and F. The proportion of each species is represented by the difference between ordinates. At all [Cl^-] the sum [A] + [B] + [C] + [D] + [E] + [F] = 100%.

10. SMITH RL. The Excretory Function of Bile. London, Chapman and Hall Publishers, 1973, p 89.
11. LEROY AF. Interactions of platinum metals and their complexes in biological systems. Environ Health Perspect 10:73–83, 1975.
12. LITTERST CL, GRAM TE, DEDRICK RL, ET AL. Distribution and disposition of platinum following intravenous administration of *cis*-diamminedichloroplatinum(II) (NSC 119875) to dogs. Cancer Res 36:2340–2344, 1976.
13. BASOLO F, GRAY HB, and PEARSON RG. Mechanism of substitution reactions of complex ions. XVII. Rates of reaction of some platinum(II) and palladium(II) complexes with pyridine. J Am Chem Soc 82:4200–4203, 1960.
14. BANERJEA D, BASOLO F, and PEARSON RG. Mechanism of substitution reactions of complex ions. XII. Reactions of some platinum(II) complexes with various reactants. J Am Chem Soc 79:4055, 1957.
15. BASOLO F, and PEARSON RG. Mechanisms of Inorganic Reactions, 2nd ed. New York, Wiley Publishers, 1967, pp 351–453.
16. GRANTHAM LF, ELLEMAN TS, and MARTIN DS, JR. Exchange of chlorine in aqueous systems containing chloride and tetrachloroplatinate(II). J Am Chem Soc 77:2965–2971, 1955.
17. ELLEMAN TS, REISHUS JW, and MARTIN DS, JR. The acid hydrolysis (aquation) of the trichloroammineplatinate(II) ion. J Am Chem Soc 80:536–541, 1958.
18. ———. Trichloroammineplatinate(II) ion. Hydrolysis, isotopic exchange of chloride and the *trans*-effect. J Am Chem Soc 81:10–18, 1959.
19. REISHUS JW, and MARTIN DS, JR. *Cis*-dichlorodiammineplatinum(II). Acid hydrolysis and isotopic exchange of the chloride ligands. J Am Chem Soc 83:2457–2462, 1961.
20. MARTIN DS, JR, and ADAMS RJ. *Trans*-dichlorodiammineplatinum(II). Acid hydrolysis and the isotopic exchange of the chloride ligands. *In* Advances in the Chemistry of Coordination Compounds (Kirchner S, ed). New York, MacMillan Publishers, 1961, p 579.
21. APRILE F, and MARTIN DS, JR. Chlorotriammineplatinum(II) ion. Acid hydrolysis and isotopic exchange of chloride ligand. Inorg Chem 1:551–557, 1962.
22. TUCKER MA, COLVIN CB, and MARTIN DS, JR. Substitution reactions of trichloroammineplatinate(II) ion and the *trans* effect. Inorg Chem 3:1373–1383, 1964.
23. LEE KW, and MARTIN DS, JR. *Cis*-dichlorodiammineplatinum(II). Aquation equilibria and isotopic exchange of chloride ligands with free chloride and tetrachloroplatinate(II). Inorg Chimica Acta 17:105–110, 1976.
24. JENSEN KA. The acid strengths of the stereoisomeric diaquodiammineplato ions. Z Anorg Chem 242:87–91, 1939.
25. PERUMAREDDI JR, and ADAMSON AW. Photochemistry of complex ions. V. The photochemistry of some square-planar platinum(II) complexes. J Phys Chem 72:414–420, 1968.

Phase I Evaluation of Piperazinedione in Patients With Advanced Cancer [1,2]

Violante Currie,* Thomas Woodcock, Charlotte Tan, Irwin Krakoff, and Charles Young [3]

SUMMARY

The effects of iv administered piperazinedione were studied in 28 evaluable adult and eight evaluable pediatric patients with advanced cancer. Piperazinedione produced predictable myelosuppression of moderate degree at dosages of 3–3.5 mg/m²/day × 5 days by iv injection. When given in this manner, nausea and vomiting did not occur. Single iv doses of 10–15 mg/m² produced mild-to-moderate nausea and vomiting and mild myelosuppression. Thrombocytopenia was more severe than leukopenia in both schedules. The drug produced comparable dose-related effects in adults and children. Although no therapeutic response was observed in the adults, a partial remission of 6 months' duration was seen in one child with Hodgkin's disease.

[Cancer Treat Rep 63:73–76, 1979]

Piperazinedione (PD) (fig 1) is an antitumor antibiotic extracted from the culture broths of *Streptomyces griseoluteus* (1). PD displays growth inhibitory activity against a variety of transplantable tumors in rodents, being particularly active against the Ehrlich ascites tumor, Taper liver tumor (ascitic form), and Walker 256 carcinosarcoma (2). In addition, PD increased survival of mice bearing L1210 leukemia by > 100% in a variety of dose schedules (3). Although this compound is believed to produce its growth inhibitory effects because it is an alkylating agent (4,5), cell lines resistant to cyclophosphamide and to BCNU have shown full sensitivity to PD (3). Studies in human tumor-derived cell lines in chick embryo egg host systems have demonstrated accumulation of cells in the G_2 phase along with progressive cell enlargement. These findings are similar to those seen with other alkylating agents (5).

A preclinical toxicologic evaluation in dogs and monkeys demonstrated drug-induced injury to the bone marrow, gastrointestinal mucosa, lymphoid tissue, and testis (6). The toxic low doses in dogs and monkeys were 0.94 and 1.12 mg/m²/day × 5 days, respectively (6). We report here the results of phase I evaluation of PD in 28 adult and eight pediatric patients.

PATIENTS AND METHODS

All patients had documented advanced cancer for which no effective conventional therapy was avail-

2,5 Piperazinedione, 3,6 -bis(5-chloro - 2- piperidyl) dihydrochloride
NSC 135, 758

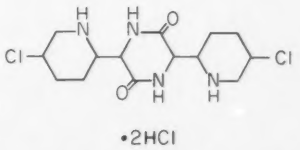


FIGURE 1.—Chemical structure of PD.

[1]Received June 24, 1977; revised Oct 25, 1978; accepted Nov 14, 1978.

[2]Supported in part by Public Health Service grants CA-08748 and CA-05826 and contract N01-CM-53804 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Clinical Chemotherapy Service, Department of Medicine (*V. Currie, T. Woodcock, I. Krakoff,* and *C. Young*) and Department of Pediatrics (*C. Tan*), Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Dr. Violante Currie, Clinical Chemotherapy Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—Clinical side effects and toxicity of PD

Side effect	Adults			Children		
	At risk			At risk		
	No. of evaluable courses	No. of evaluable patients	Total No. of courses with toxic effects	No. of evaluable courses	No. of evaluable patients	Total No. of courses with toxic effects
Nausea and vomiting	45	28	2	15	8	4
Alopecia	45	27	2	15	8	0
Stomatitis	45	27	1	15	8	0
Rash	45	28	1	15	8	0
Fever	45	28	2	15	8	0

TABLE 2.—Leukopenia produced by PD: dose-effect relationships

Dose (mg/m^2)	No. of evaluable courses	No. of evaluable patients	No. of courses of wbc nadir ($\times 10^3/mm^3$) of—		
			> 4.0	2.0–3.9	< 2.0
Daily × 5 dose schedule					
Adults					
0.3–0.9 × 5	7	7	7	0	0
2.0 × 5	5	4	2	2	1
3.0–3.5 × 5	11	10	3	4	4
4.0–5.0 × 5	5	5	0	2	3
Single-dose schedule					
Adults					
4.0	1	1	1	0	0
10.0–12.0	14	6	9	3	2
15.0	1	1	0	0	1
Children					
5.0–8.0	3	3	2	1	0
10.0–12	8	3	5	3	0
15.0	4	3	0	1	3

able. Twenty-six of the 28 adult patients and all of the eight pediatric patients had previously received cytotoxic chemotherapy. Informed consent for the drug trial was obtained from all participants or guardians.

PD was provided as a lyophilized powder by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). The medication was reconstituted with water just prior to administration at a concentration of 1 mg/ml and given iv. Two dose schedules were investigated: (*a*) daily injections × 5 days in adult patients, and (*b*) single doses every 7–14 days in adult and pediatric patients. When patients received repeated courses of PD, all hematologic parameters had returned to control values for at least 1 week prior to starting another course.

RESULTS AND DISCUSSION

A phase I evaluation of PD has been carried out in 26 adults by a daily dose (× 5 days) administration and in eight adults and eight children by a single-dose (every 7–14 days) administration. The subjective and objective physical manifestations of drug toxicity produced by PD were negligible (table 1). In the adult patients, nausea and vomiting occurred only in those receiving single doses > 10 mg/m^2. Hematopoietic toxicity was dose-limiting. With rare exceptions, thrombocytopenia was more marked than leukopenia. The dose-effect relationships between the quantity of drug administered and the hematopoietic toxicity are summarized in tables 2 and 3.

On the daily schedule, in 21 evaluable courses in adults in the dose range of 2–5 mg/m^2/day × 5 days,

TABLE 3.—Thrombocytopenia produced by PD: dose-effect relationships

Dose (mg/m^2)	No. of evaluable courses	No. of evaluable patients	No. of courses of platelet nadir ($\times 10^3/mm^3$) of—		
			> 150	80–150	< 80
Daily × 5 dose schedule					
Adults					
0.3–0.9 × 5	6	7	6	1	1
2.0 × 5	4	4	2	1	1
3.0–3.5 × 5	11	10	1	3	7
4.0–5.0 × 5	5	5	0	0	5
Single-dose schedule					
Adults					
4.0–5.0	1	1	1	0	0
10.0–12.0	14	6	9	3	1
15.0	1	1	0	0	1
Children					
5.0–8.0	3	3	2	1	0
10.0–12.0	8	3	7	1	0
15.0	4	3	0	0	4

TABLE 4.—Time to wbc and platelet count nadirs

	Daily dose regimen			Single-dose regimen					
	Adults			Adults			Children		
	No. of courses	Median treatment day to—		No. of courses	Median treatment day to—		No. of courses	Median treatment day to—	
		Nadir	Recovery		Nadir	Recovery		Nadir	Recovery
Leukopenia*	18	18	34	6	14	28	8	14	25
Thrombocytopenia†	19	16	33	5	15	26	6	19	25

*Wbc count nadir ≤ $3.9 \times 10^3/mm^3$.
†Platelet count nadir ≤ $150 \times 10^3/mm^3$.

the time to the wbc and platelet count nadirs was essentially the same (18 and 16 days, respectively) with recovery also being equal (34 and 33 days, respectively). On the single-dose schedule, the nadirs of the wbc and platelet counts in adults and children were also essentially equal (table 4). The severity of the hematopoietic toxicity did not seem to affect the time to nadir or recovery of the wbc or platelet counts.

Whether by daily dose × 5 days in adults or by single dose every 7–14 days in adults and children, a course of 5 mg/m^2 of PD did not produce consistent leukopenia or thrombocytopenia; it was the highest nontoxic dose studied for adults. When given in five daily equal doses, a course of 15–17.5 mg/m^2 produced significant thrombocytopenia in ten of ten adult patients and leukopenia in eight. This would appear to be a satisfactory dose for phase II trials. A dose escalation up to 20–25 mg/m^2/course produced leukopenia and thrombocytopenia in all patients.

Although explored only briefly, it appears that a single dose, corresponding to the same total dose given on a daily × 5 regimen, would produce about the same myelosuppression as the divided-dose schedule. These observations are in accord with the extended evaluation of the single-dose route conducted by Gottlieb and colleagues who suggested a phase II schedule of 12–15 mg/m^2 at 3–4-week intervals in solid tumors and 24 mg/m^2 for patients with leukemia (3). The data presented here provide further support for the appropriateness of the dose of 12 mg/m^2 utilized in phase II studies in breast and renal cancer (9,10), melanoma (11), and malignant lymphoma (12).

Although the determination of therapeutic activity was not the principal purpose of this toxicologic study, a partial remission of 6 months' duration was seen in one child with Hodgkin's disease; no therapeutic effects were seen in adults.

REFERENCES

1. GITTERMAN CO, RICKES EL, WOLF DE, ET AL. The human tumor-egg host system. IV. Discovery of a new antitumor agent compound 593 A. J Antibiot (Tokyo) 23:305–310, 1970.
2. TARNOWSKI GS, SCHMID FA, HUTCHISON DJ, ET AL. Chemotherapeutic effects of compound 593A (NSC-135758) on mouse leukemias and some transplanted animal tumors. Cancer Chemother Rep 57:21–27, 1973.
3. SCHABEL FM, JR, TRADER MW, LASTER WR, JR, ET AL. Studies with 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-,dihydrochloride. III. Biochemical and therapeutic effects in L1210 leukemias sensitive and resistant to alkylating agents: comparison with melphalan, cyclophosphamide, and BCNU. Cancer Treat Rep 60:1325–1333, 1976.
4. BROCKMAN RW, SHADDIX SC, WILLIAMS M, ET AL. Studies with 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride. II. Effects on macromolecular synthesis in cell culture and evidence for alkylating activity. Cancer Treat Rep 60:1317–1324, 1976.
5. WHEELER GP, BONO VH, BOWDON BJ, ET AL. Studies with 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride. I. Cell kinetic and biologic effects in cultured L1210, human epidermoid No. 2, and Adenocarcinoma 755 cells. Cancer Treat Rep 60:1307–1316, 1976.
6. FOLK RM, PETERS AC, PAVKOV KL, ET AL. Preclinical toxicologic evaluation of 2,5-piperazinedione, 3,6-bis(5-chloro-2-piperidyl)-, dihydrochloride (NSC-135758) in dogs and monkeys. Cancer Chemother Rep Part 3, vol 5(1):37–41, 1974.
7. DOLLINGER MR, BURCHENAL JH, KREIS W, ET AL. Analogs of 1-β-D-arabinofuranosylcytosine. Studies on mechanism of Burkitt's cell cultures and mouse leukemia and in vitro deamination studies. Biochem Pharmacol 16:689–706, 1967.
8. GOTTLIEB JA, FREIREICH EJ, BODEY GP, ET AL. Preliminary clinical evaluation of piperazinedione (P), a new crystalline antibiotic. Proc Am Assoc Cancer Res and ASCO 16:86, 1975.
9. PALMER RL, SAMAL BA, VAUGHN CB, ET AL. Phase II evaluation of piperazinedione in metastatic breast carcinoma. Cancer Treat Rep 61:1711–1712, 1977.
10. PASMANTIER MW, COLEMAN M, KENNEDY BJ, ET AL. Piperazinedione in metastatic renal carcinoma. Cancer Treat Rep 61:1731–1732, 1977.
11. AL-SARRAF M, THIGPEN T, GROPPE CW, ET AL. Piperazinedione in patients with advanced malignant melanoma: a Southwest Oncology Group study. Cancer Treat Rep 62:1101–1103, 1978.
12. JONES SE, TUCKER WG, HAUT A, ET AL. Phase II trial of piperazinedione in Hodgkin's disease, non-Hodgkin's lymphoma, and multiple myeloma: a Southwest Oncology Group study. Cancer Treat Rep 61:1617–1621, 1977.

Phase II Study With Sequential L-Asparaginase and Methotrexate in Advanced Refractory Breast Cancer [1,2,3]

Hwee-Yong Yap,* Robert S. Benjamin, George R. Blumenschein, Gabriel N. Hortobagyi, Charles K. Tashima, Aman U. Buzdar, and Gerald P. Bodey [4,5]

SUMMARY

The combination of sequential L-asparaginase and methotrexate (MTX) was evaluated in 33 patients with advanced refractory breast cancer. There were nine partial responses and one complete response, giving an overall response rate of 30% and a median duration of response of 8 months. Five of 17 patients (28%) who had received prior MTX at doses of < 50 mg/m^2 responded. Toxicity was acceptable. Moderate-to-severe stomatitis occurred in most patients and was the dose-limiting factor. Myelosuppression was minimal until the dose of MTX was escalated to ≥ 180 mg/m^2. The maximum tolerated dose of MTX was 280 mg/m^2 and the median toxic dose was 220 mg/m^2. These data indicate a selective "rescue" from MTX damage to normal target tissue by L-asparaginase. The antitumor effect observed even in patients who had been previously exposed to conventional doses of MTX suggests a possible improved therapeutic index of MTX given sequentially with L-asparaginase in this combination.

[Cancer Treat Rep 63:77–83, 1979]

Combination chemotherapy has significantly improved the response rate, duration of response, and survival of patients with advanced metastatic breast cancer. Unfortunately, despite these encouraging advances, the median duration of response continues to be short and is at best approximately 10–15 months (1–3). There is, therefore, a need to develop new effective programs to be utilized in the event of primary multiple-drug failures. Based on the work of Capizzi and others (4–6) on the use of sequential L-asparaginase and methotrexate (MTX), we have investigated this combination in patients with advanced refractory stage IV breast cancer. The combination of L-asparaginase and MTX can be both synergistic and antagonistic depending on the dose and schedule employed. L-Asparaginase is thought to inhibit protein-dependent DNA synthesis and can, therefore, rescue normal hemopoietic cells from the S-phase specific toxicity of MTX allowing for an enhanced therapeutic index of MTX. If L-asparaginase is given just before MTX, the cytotoxic effect of MTX is abrogated or considerably reduced. Conversely, if the interval between L-asparaginase administration and that of subsequent MTX is 7–14 days, there can be enhancement of the therapeutic effect of MTX (5).

MATERIALS AND METHODS

Forty patients with advanced metastatic breast cancer who had become resistant to various adriamycin-containing regimens were entered in this

[1] Received June 26, 1978; revised Oct 11, 1978; accepted Oct 31, 1978.

[2] Presented in part at the 14th Annual Meeting of The American Society of Clinical Oncology, Washington, DC, Apr 3–4, 1978.

[3] Supported by contract N01-CM-57042 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[4] Medical Breast Service, Department of Medicine (*H.-Y. Yap, G. R. Blumenschein, G. N. Hortobagyi, C. K. Tashima,* and *A. U. Buzdar*), and Department of Developmental Therapeutics (*R. S. Benjamin* and *G. P. Bodey*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston. Dr. R. S. Benjamin is a Junior Faculty Fellow, American Cancer Society.

[5] We wish to thank Audrey Faye Grubbs for her technical assistance.

* *Reprint requests to:* Hwee-Yong Yap, MD, Medical Breast Service, Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 1.—Sequential L-asparaginase and MTX treatment plan

Day	Drug	Dose and schedule
1	MTX	50 mg/m² iv/im, followed in 3 hrs by L-asparaginase at 10,000 units/m² im
14	MTX	120 mg/m² iv/im
15	L-Asparaginase	10,000 units/m² im
29	MTX	160 mg/m² iv/im
30	L-Asparaginase	10,000 units/m² im
43	MTX	Repeat cycle every 14 days
44	L-Asparaginase	

study. Seven patients were found to be inevaluable for the following reasons: two patients died before completing their first course of this sequential combination, two did not have their doses of MTX escalated to > 50 mg/m² and were regarded as major protocol violations, and three were lost to followup. The treatment program (table 1) modified from that suggested by Capizzi (4) consisted of L-asparaginase at a dose of 10,000 units/m² given im on Day 1; 14 days later MTX at a dose of 120 mg/m² was given im/iv followed by L-asparaginase 24 hours later. This 2-day course was repeated at 14-day intervals. To ensure that potentially effective treatment would not be delayed, all patients received an "unprotected" low dose of MTX at 50 mg/m² 3 hours prior to the initiation of the sequential program. The L-asparaginase dose remained constant while the MTX dose was escalated by increments of 40 mg/m²/course to tolerance (lowest granulocyte count not < 1000/mm³ and/or lowest platelet count not $< 100{,}000$/mm³ without severe stomatitis limiting food intake). If unacceptable toxicity occurred, the next dose of MTX was then reduced by 20 mg/m² and was not given until all evidence of toxicity had cleared. If resolution of MTX toxicity did not occur until > 14 days after the prior L-asparaginase dose, the next dose of MTX was then withheld until 14 days after a repeat dose of L-asparaginase.

Patients were eligible for this therapy only if they had a serum creatinine of < 1.5 mg/100 ml with a normal BUN and a creatinine clearance level of > 60 ml/minute. Other prerequisites for entry in this study included evidence of measurable disease and adequate bone marrow and liver function. A complete history and physical examination, cbc, urinalysis, liver and renal function tests, chest roentgenograms, selected bone roentgenograms, and liver and bone scans were done prior to the initiation of therapy and were repeated at regular intervals during therapy. Written informed consent was obtained from all patients. In this study, a complete remission was defined as the complete disappearance of all objective and subjective evidence of disease including complete recalcification of bone lesions. Partial remission was defined as a $\geq 50\%$ reduction in the product of the diameter of measurable lesions including recalcification of osteolytic lesions. Patients with a $< 50\%$ reduction or a $< 25\%$ increase in tumor size for a minimum period of 2 months were considered to have stable disease. Progressive disease was defined as a $> 25\%$ increase in existing tumors or the appearance of any new lesions. The duration of remission was determined from the date of achieving remission to the date of relapse or progression. The duration of therapy was measured from the start of chemotherapy to the date that chemotherapy was discontinued. Survival was measured from the start of treatment to the date of death or last followup examination.

The Kaplan and Meier method was used to calculate and plot remission and survival curves (7) and a generalized Wilcoxon test with a two-tailed analysis was used to test differences between remission and survival curves (8).

RESULTS

Among the 33 evaluable patients, there were nine partial responses and one complete response, giving an overall response rate of 30% and a median duration of response of 35 weeks (range, 4+ to 49 weeks). Twelve patients achieved stability of their disease and the median duration of stable disease was 13 weeks, while 11 patients continued to progress in spite of this treatment. The duration of therapy by response status is shown in figure 1. The median duration of therapy was 37 weeks for responders compared with 14 weeks for those patients with stable disease and 7 weeks for those who failed to respond ($P < 0.05$). The survival curves are shown in figure 2. The median survival time for the responders has not been reached but 60% of the patients are alive at 52 weeks, compared with a

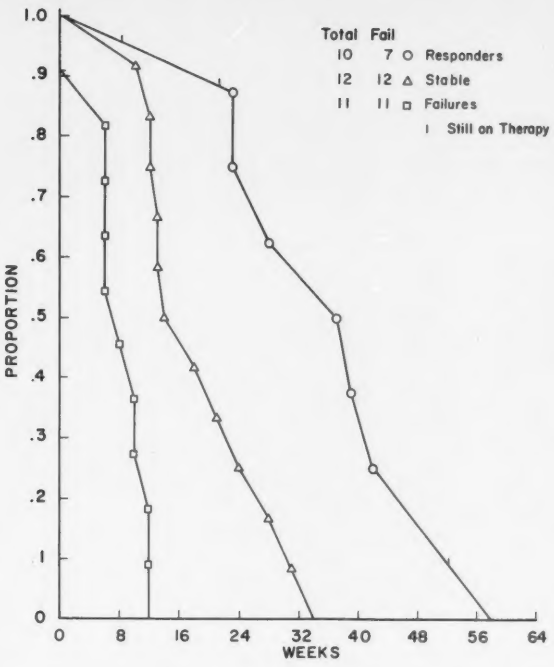


FIGURE 1.—Duration of therapy by response status.

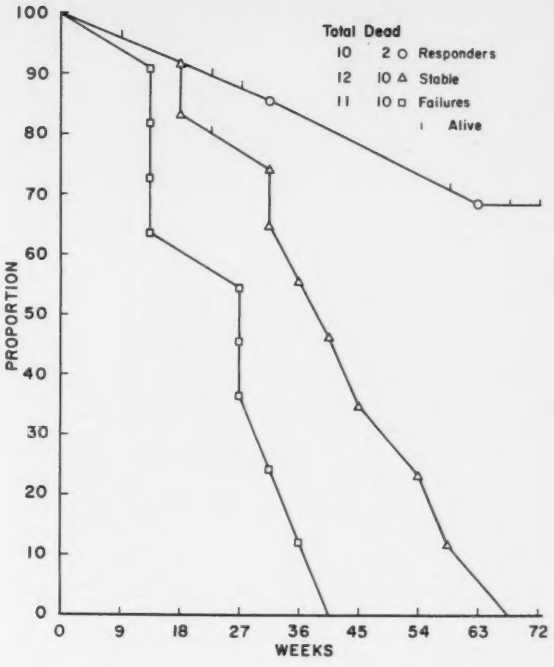


FIGURE 2.—Survival curves for all patients.

median survival time of 38 weeks for patients with stable disease ($P = 0.01$). Patients with stable disease survived significantly longer ($P = 0.01$) than those whose disease failed to respond, and the median survival time for failures was 27 weeks. Table 2 summarizes the patients' pretreatment characteristics according to response status. All patients had had extensive prior chemotherapy with a median duration of 12 months (range, 6–16 months). There was no apparent relationship between disease-free interval, menopausal status, duration of prior therapy, dominant disease sites, number of organ sites involved, and response to this treatment. The sites of response are shown in table 3. Seventeen patients had prior exposure to MTX. All patients progressed while receiving a combination of cyclophosphamide, MTX, and 5-fluorouracil (5-FU) given as maintenance therapy for the 5-FU, adriamycin, and cyclophosphamide program (1–3). The MTX dose was 30 mg/m²/day on Days 1 and 8 every 21 days. Table 4 compares those patients who had had prior MTX therapy to those who had not had prior exposure to MTX. No difference was found in response rate or duration of response achieved by these two groups of patients.

Toxicity

Tables 5 and 6 outline the myelosuppression and stomatitis observed at various MTX dose levels. In most instances, myelosuppression was minimal until the doses of MTX were escalated to ≥ 160 mg/m². The lowest granulocyte and platelet counts were encountered between Days 6 and 10 of each course. Recovery was rapid and most patients were able to receive courses of treatment every 14 days. The incidence of severe stomatitis causing functional disturbance increased as the dose of MTX was increased, and for most patients this was the dose-limiting factor. The maximum tolerated dose of MTX was 280 mg/m² and the median toxic dose was 220 mg/m². This treatment was well-tolerated with acceptable toxic effects except for one drug-related death from septicemia, two cases of transient hyperglycemia and liver dysfunction, and one case of abdominal pain without proven pancreatitis. There was no acute anaphylactic reaction related to im L-asparaginase. Nine patients developed myelosuppression with or without moderate stomatitis when 50 mg/m² of MTX (unprotected) was given 3 hours prior to the initiation of the L-asparaginase

TABLE 2.—Pretreatment patient characteristics

	CR and PR*	Stable disease	Failure
No. of patients	10	12	11
Age in yrs			
Range	34–65	38–63	26–62
Median	55	53	45
Disease-free interval in mos			
Range	0–34	2–75	0–108
Median	9	16	11
No. of patients with—			
Menopausal status			
Premenopausal	3	3	3
Perimenopausal	1	1	2
Postmenopausal	5	8	4
Unknown	1	0	2
Prior chemotherapeutic regimens†			
VAC/FAC	10	12	11
Other 2nd-line therapy	2	6	1
MTX (CMF/FUM)	5	6	6
Dominant disease sites			
Soft tissue	2	2	2
Bone	3	4	2
Visceral	5	6	7
No. of organ sites involved			
1	2	5	4
2	4	6	4
3	1	1	3
4	3	0	0
> 4	0	0	0
Duration of prior therapy in mos			
Hormonal	16 (1 patient)	1–57 (5 patients)	1+ to 12 (2 patients)
Chemotherapy			
Range	6–25	3–41	4–25
Median	10	14	12
Total			
Range	6–35	6–60	6–27
Median	10	15	12

*CR = complete response; PR = partial response.

†VAC = vincristine, adriamycin, and cyclophosphamide; FAC = 5-FU, adriamycin, and cyclophosphamide; CMF = cyclophosphamide, MTX, and 5-FU; FUM = 5-FU and MTX.

TABLE 3.—Response rate according to sites of metastases*

Sites of metastasis	No. of patients		Response (%)
	CR and PR	Total	
Breast + soft tissue	5	15	33
Lymph nodes	4	7	57
Bone	5	16	31
Lung	2	12	16
Pleura	0	5	0
Liver	1	3	33
Bone marrow	0	2	0
Other	1	1	100

*Differences are not statistically significant.

TABLE 4.—Response to therapy according to prior MTX experience*

	No. of patients	
	Prior MTX	No prior MTX
Total evaluable	17†	16
CR and PR	5 (29%)	5 (31%)
Stable disease	6	6
Failure	6	5

* Differences are not statistically significant.
† All patients progressed with CMF (MTX at 30 mg/m²/day on Days 1 and 8 q21days).

TABLE 5.—Hematologic toxic effects

MTX (mg/m²)	No. of courses	Lowest counts (cells × 10³/mm³)		
		Total wbcs	Granulocytes	Platelets
50	21	3.9	2.2	225
		1.5–7.4	0.6–7.0	50–318
120	24	3.9	3.1	252
		2.0–8.5	0.8–7.0	82–440
160	20	3.5	1.4	188
		0.8–8.6	0.4–7.2	70–318
200	15	2.6	1.8	281
		2.1–5.5	0.6–4.5	85–510
240	12	3.2	1.8	164
		1.1–7.8	0.2–5.5	52–358
260	5	1.3	0.7	110
		1.0–1.5	0.5–0.8	26–180
280	2	2.1, 4.9	1.8, 3.6	210, 358

TABLE 6.—Incidence of stomatitis related to dose of MTX

MTX (mg/m²)	No. of courses	No. of courses (%) with—	
		Moderate stomatitis*	Severe stomatitis†
50	33	2 (6)	0
120	38	0	0
160	48	3 (6)	1 (2)
180	51	1 (2)	1 (2)
200	72	6 (8)	3 (4)
240	31	2 (6)	2 (6)
280	12	1 (8)	2 (16)
300	2	1 (50)	1 (50)

* Moderate stomatitis = presence of multiple oral mucosal ulcerations with some limitation of food intake.
† Severe stomatitis = presence of multiple oral mucosal ulcerations with denudation causing marked limitation of food intake.

TABLE 7.—Subsequent experience with protected MTX dose escalation in 9 patients who developed myelosuppression at 50 mg/m² "unprotected-dose" MTX*

MTX (mg/m²)	No. of courses	Lowest counts (cells × 10³/mm³)	
		Granulocytes	Platelets
50	9	0.9 (0.6–1.2)	142 (50–201)
120	10	1.7 (1.0–2.1)	154 (82–182)
160	16	1.9 (0.4–5.0)	188 (70–322)
200–240	8	1.4 (0.8–2.0)	157 (113–281)

*Paired t tests revealed a significant reduction in the degree of granulocytopenia ($P < 0.05$) when MTX was given in the sequential manner with L-asparaginase in escalating doses: 50 mg/m² vs 120 mg/m², $P < 0.01$; 50 mg/m² vs 160 mg/m², $P < 0.04$.

and MTX combination; table 7 summarizes their subsequent experience with escalating doses of MTX given in the sequential manner with L-asparaginase. All nine patients had a significant reduction in the degree of granulocytopenia ($P < 0.05$) and were able to tolerate 2.5–4 times the unprotected low-dose MTX when given sequentially with L-asparaginase.

DISCUSSION

A 30% response rate was observed with this sequential L-asparaginase and MTX combination in patients with refractory stage IV breast cancer. This is not significantly better than the reported response rate with MTX alone (9,10) and is comparable to our experience with high-dose MTX (2–7.5 g/m²) and citrovorum factor rescue.[6] However, the antitumor effect that was observed, even in patients who had been previously exposed to conventional doses of MTX, suggests a possibly improved therapeutic index of MTX given sequentially with L-asparaginase in this combination. Furthermore, our data suggest that this regimen is suitable for use as salvage therapy for those patients who have previously demonstrated resistance to MTX at doses < 50 mg/m². Although there is a wide variability of individual tolerances to MTX in cancer patients (11), doses > 80 mg/m² are, in most instances, considered to be potentially hazardous unless given with citrovorum factor rescue. In one study (11), the median toxic dose of MTX was 120 mg/m² when given as a single 1-hour iv infusion. In the present study, the median toxic dose of MTX was 220 mg/m² when given sequentially with L-asparaginase. Furthermore, the nine patients who developed myelosuppression with or without stomatitis with the initial unprotected dose of MTX of 50 mg/m² were subsequently able to tolerate 2.5–4 times the unprotected dose of MTX when combined with L-asparaginase given in the sequential manner. These data tend to further substantiate the initial observation by Capizzi that L-asparaginase can act as a selective rescue to protect normal host target tissues against MTX toxicity. The ease of administering this treatment program and the relative lack of toxicity together with the enormous cost of high-dose MTX at 2–7 g/m² with citrovorum factor rescue should make this treatment regimen a worthwhile alternative for the treatment of MTX-sensitive neoplasms.

[6] Yap H-Y, et al. Data to be published.

REFERENCES

1. BLUMENSCHEIN GR, CARDENAS JO, FREIREICH EJ, ET AL. FAC chemotherapy for breast cancer. Proc Am Assoc Cancer Res and ASCO 15:193, 1974.
2. GUTTERMAN JU, CARDENAS JO, BLUMENSCHEIN GR, ET AL. Chemoimmunotherapy of disseminated breast cancer: prolongation of remission and survival. Br Med J 2:1222–1225, 1976.
3. HORTOBAGYI GN, GUTTERMAN JU, BLUMENSCHEIN GR, ET AL. Combination chemoimmunotherapy of metastatic breast cancer with 5-fluorouracil, adriamycin, and cyclophosphamide, and BCG. Cancer, 1978. In press.
4. CAPIZZI RL. Schedule-dependent synergism and antagonism between methotrexate and L-asparaginase. Biochem Pharmacol 23 (suppl 2):151–161, 1974.
5. CHLOPKIEWICZ B, and KOZIOROWSKA J. Role of amino acid depletion in combined treatment of neoplastic cells with methotrexate and L-asparaginase. Cancer Res 35:1524–1529, 1975.
6. CAPIZZI RL. Improvement in the therapeutic index of methotrexate (NSC-740) by L-asparaginase (NSC-109229). Cancer Chemother Rep Part 3, vol 6(1):37–41, 1975.
7. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.
8. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–223, 1965.

9. ANDREWS NC, and WILSON WL. Phase II study of methotrexate (NSC-740) in solid tumors. Cancer Chemother Rep 51:471–474, 1967.
10. VOGLER WR, FURTADO VP, and HUGULEY CM. Methotrexate for advanced cancer of the breast. Cancer 21:26–30, 1968.
11. HANSEN HH, SELAWRY OS, HOLLAND JF, ET AL. The variability of individual tolerance to methotrexate in cancer patients. Br J Cancer 25:298–305, 1971.

Enzymatic Assay for the Antitumor Agent *N*-(Phosphonacetyl)-L-aspartic Acid (PALA) [1,2]

Jacqueline Friedman, E. Colleen Moore,* Stephen W. Hall, and Ti Li Loo [3]

SUMMARY

A method is described for the assay of *N*-(phosphonacetyl)-L-aspartic acid in plasma or urine, based on inhibition of partially purified aspartate carbamoyltransferase from rat liver. Concentrations of *N*-(phosphonacetyl)-L-aspartic acid as low as 0.1 μg/ml can be detected. Plasma disappearance and urinary excretion curves from one patient are shown as examples of the application of the method.

[Cancer Treat Rep 63:85–88, 1979]

N-(Phosphonacetyl)-L-aspartic acid (PALA) is an inhibitor of the enzymatic transfer of the carbamoyl group of carbamoyl phosphate to L-aspartic acid to give carbamoyl aspartate (1). This reaction, an early step in the de novo biosynthesis of pyrimidine nucleotides, is mediated by aspartate carbamoyltransferase (2) (ACTase, EC 2.1.3.2). Because of its activities against various transplantable murine neoplasms (3), particularly certain solid tumors, PALA is currently in clinical trial as a potential anticancer agent at our institution. To study the clinical pharmacokinetics of PALA, it is desirable to have a sensitive and specific assay for the agent in biologic specimens. We describe an enzymatic method for the assay of PALA that was developed in our laboratories based on its competitive inhibition of ACTase.

MATERIALS AND METHODS

Materials

L-Aspartic acid-UL-^{14}C (specific activity, 192 millicuries/mmol), purchased from New England Nuclear Corp, was purified by adsorption and elution from Dowex 50 as described by Porter et al (4), and was diluted to 0.25–0.38 millicurie/mmol. Other chemicals, reagents, and suppliers are: carbamoyl phosphate, dilithium salt from Sigma Chemical Co; Hepes buffer from Calbiochem; Dowex 50W-X8 (hydrogen form) from BioRad; Aquasol, a universal liquid scintillation fluid, from New England Nuclear Corp; and gel filtration medium Ultrogel AcA22 from LKB.

Enzyme Preparation

The ACTase preparation which was used for the experiments reported here was prepared from the livers of rats bearing Novikoff ascites hepatoma. The method of Mori et al (5) was followed through the second ammonium sulfate precipitation. The precipitate was then subjected to gel filtration on Ultrogel AcA22, dialyzed against 2 mM phosphate containing 0.5 mM EDTA and 1 mM dithiothreitol, frozen in small aliquots, and stored at –80°C. The original specific activity of the preparation was 2.7 nmols/minute/mg. Protein was estimated using the BioRad kit of dye reagent and γ-globulin standard. A number of other enzyme batches, prepared from the livers of normal or tumor-bearing rats by several variations of this procedure, have been used in earlier and later work with similar results.

Enzyme Assay

ACTase was assayed by isolation of ^{14}C-carbamoyl aspartate as described by Porter et al (4) and

[1]Received June 27, 1978; revised Nov 7, 1978; accepted Nov 13, 1978.

[2]Supported by contract N01-CM-53773 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Departments of Biochemistry and Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

Reprint requests to: E. Colleen Moore, PhD, Department of Biochemistry, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

modified by Hoogenraad et al (6). The separation depends on the binding of aspartate, through its α-amino group, to the cation exchanger Dowex 50. Unless otherwise specified, all samples contained 0.2 mM carbamoyl phosphate, 0.4 mM ^{14}C-aspartic acid, 50 mM Hepes (pH 7.4), and 25 μl (0.05 mg) of ACTase, in a total volume of 175 μl; however, the enzyme was omitted from the blank.

The reaction was started by adding 50 μl of a mixture of buffer and substrates to a tube containing plasma, PALA, ACTase, and water. After each tube was incubated for 30 minutes at 37°C, 1.5 ml of 0.2 M acetic acid was added to stop the reaction. The mixtures were passed through 0.7 × 2.5-cm columns of Dowex 50 (H^+). The effluents, combined with three washes of 1 ml of water, were collected in counting vials. Twelve ml of Aquasol was added to each vial and the clear gels were counted in a Packard 2650 liquid scintillation spectrometer. Any quenching was automatically corrected, and counts were automatically converted to disintegrations per minute (dpm).

Activity was expressed as nmols converted in 30 minutes in the inhibited sample divided by nmols converted in 30 minutes in the controls. The radioactivity in the blank without ACTase was subtracted from that of each sample before calculating the nmols converted. To achieve linearity, the standard curve was plotted as the reciprocal of the enzyme activity versus PALA concentration (7). This plot gives a straight line provided the substrate concentration is constant. Each standard curve included four concentrations of PALA in duplicate. The line was fitted by the least-squares method.

Plasma and Urine Samples

Blood was drawn from three healthy subjects, with heparin (14.3 units/ml of blood) as the anticoagulant. Plasma was separated by centrifugation and stored frozen until used. Urine was obtained from two healthy subjects and stored frozen until used. Plasma and urine samples from a patient who received a dose of 1600 mg/m^2 of PALA in a 1-hour iv infusion were treated similarly.

RESULTS

Enzyme and Product

The activity of the enzyme preparation was linear with time for at least 30 minutes, and with enzyme protein up to 0.1 mg. The activity did not vary significantly with carbamoyl phosphate concentration from 0.1 to 3 mM , which is not surprising since the K_m is 3.5 μM (2). Mammalian ACTase is not subject to allosteric effects as is the bacterial enzyme (2). Under our standard conditions, the incubation of 25 μl of enzyme (0.05 mg) for 30 minutes without inhibitor usually gave about 4 nmols of carbamoyl aspartate, or about 3000 dpm over a blank of about 100 dpm (< 3% of the total dpm). An occasional vial of enzyme gave lower activity, but the standard inhibition curve was similar. Vials were not refrozen after thawing; any unused enzyme was discarded. Blanks without carbamoyl phosphate were not run routinely. The partially purified ACTase by itself showed negligible activity under these conditions.

The stability of the product was tested under the conditions of incubation and separation. There was no change in samples which were allowed to stand in acetic acid for 1 hour at room temperature before application to the Dowex 50 columns. A large excess of PALA was added to other samples, after incubation with ACTase, to stop the ACTase reaction; the incubation was continued for 30 minutes at 37°C before adding acetic acid and applying it to the columns. With 30 μl of plasma, no significant destruction of product occurred since the product radioactivity did not vary > 5%. (This does not deny the probability that some or all of the carbamoyl aspartate which is formed is converted to dihydroorotate by the enzyme complex [5,6,8], since dihydro-orotate would also pass through Dowex 50 and be counted as product in our assay.)

Standard Curve

The standard curve was linear up to about 8 ng of PALA per incubation tube (0.8 μM in the incubation mixture). With the substrate concentrations of 0.2 mM carbamoyl phosphate and 0.4 mM aspartate, and with no more than 5 μl of plasma or urine per assay tube, it was reproducible (fig 1). For the set of eight standard curves determined with this batch of enzyme, the mean (and SD) of the slopes was 0.55 (0.07), and the mean (and SD) of the intercept was 1.05 (0.06). The variability was much greater with one half of these substrate concentrations (data not shown).

Because of interference with large amounts of plasma and urine (*see* below), pretreatment plasma or urine from the same patient was required to make up a constant volume in all assay tubes, including controls, blanks, and PALA standards. For the best results, all samples from the same patient should be assayed at the same time.

Since some individual vials of ACTase were low in activity, we tested for variation in inhibition by PALA with enzyme concentration. There was no

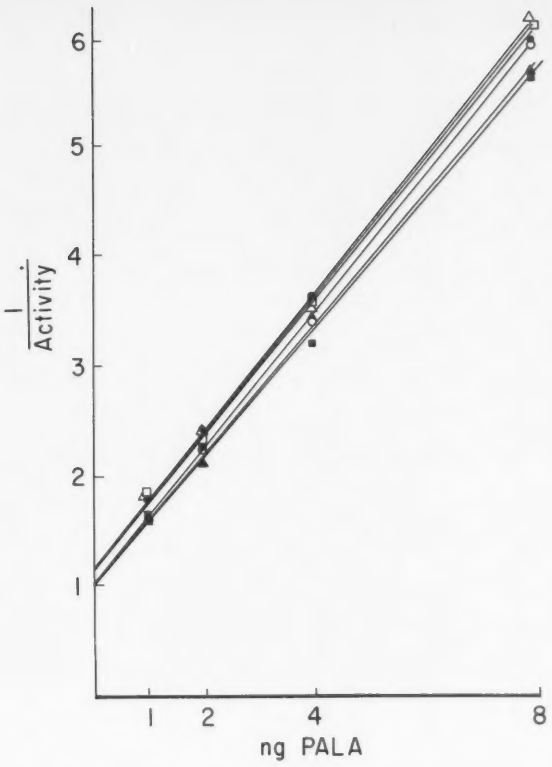


FIGURE 1.—Abscissa = ng of PALA/incubation tube. Ordinate = reciprocal of enzyme activity expressed as fraction of uninhibited activity. Each symbol = a separate standard curve; each point is the average of 2 assay tubes. ○, ▲, ● = plasma added; □, △, ■ = urine added. The lines were fitted by least-squares regression analysis.

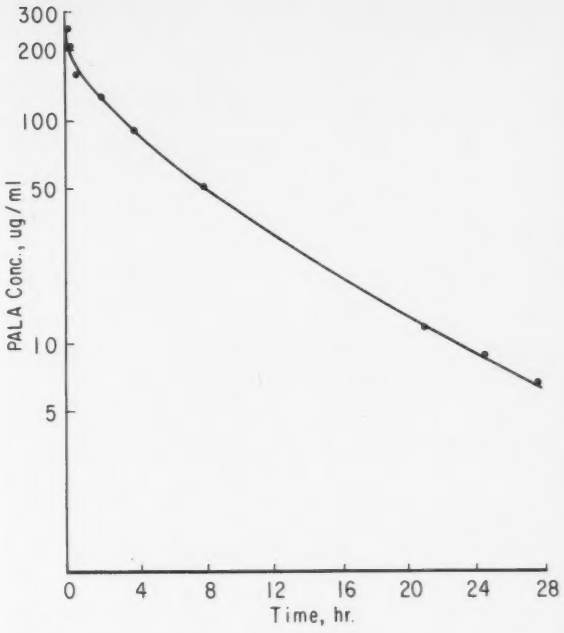


FIGURE 2.—Plasma disappearance of PALA in a patient after an iv infusion of 1600 mg/m². Abscissa = time in hrs. Ordinate = μg/ml of PALA. The curve is expressed by the equation: $C_p = 107.5e^{-1.25t} + 151.5e^{-0.116t}$, where C_p is plasma concentration and t is time in hrs.

change in inhibition when the enzyme level was reduced fourfold, from 4 to 1 nmols converted in the controls.

Interference

The addition of 20–120 μl of plasma or urine to the reaction mixture had three effects: (*a*) the radioactivity in the blank (no ACTase) was increased up to 200%, (*b*) the apparent enzyme activity was decreased as much as 25%, and (*c*) the apparent inhibition by PALA was lower. There was significant variation among different samples of plasma and even more variation among urine samples. The high sensitivity of the assay, however, enabled us to decrease the plasma or urine volume to ≤ 10 μl/assay tube. At this concentration, the effects on the blank radioactivity were negligible. With 10 μl of plasma, the other effects were reduced sufficiently to be tolerable, < 10% depression of control activity and about 15% decrease in sensitivity. With ≤ 1 μl, the interference was completely removed.

We have not made a serious effort to identify the causes of the interference, but possible explanations include deamination of aspartate (by aspartate aminotransferase and 2-oxoglutarate), dilution of labeled substrate by unlabeled aspartate, and, less likc¹y, competition by carbamoyl phosphate. In order to apply the assay to tissue extracts we are now investigating possible methods of removing interfering materials.

When the body fluid is limited to 10 μl/assay, the lowest point on the standard curve, 1 ng of PALA, represents 1 ng in 10 μl, 0.1 μg/ml, or 0.38 μM. For three successive assays (six samples) under these conditions, this amount of PALA gave a mean of 59.3% (SD = 7.8) of the control activity, an inhibition which is easily detectable. In the presence of 5 μl of plasma, the corresponding mean was 64.8% (SD = 6.0, 12 samples), and in the presence of 0.5 μl of plasma, the corresponding mean was 56.5% (SD = 9.8, ten samples).

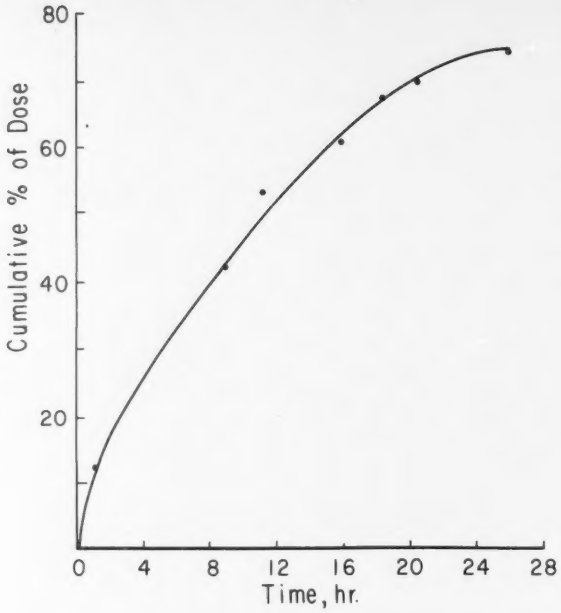


FIGURE 3.—Cumulative urinary excretion of PALA in a patient after an iv infusion of 1600 mg/m². Abscissa = time in hrs. Ordinate = cumulative % of the administered dose.

Patient Study

A patient received a dose of 1600 mg/m² of PALA in a 1-hour iv infusion. At this dose, the initial PALA concentration in the plasma was high enough to require a 1:1000 dilution before assay. We were able to measure a 3-decade decrease over 24 hours using no more than 1 μl of plasma per assay (fig 2). The initial plasma half-life in this patient was about 33 minutes; the second plasma half-life was 6.0 hours, and the concentration × time value that was estimated for the complete elimination of PALA from the plasma was 1.39 g/hr/liter. The apparent volume of distribution of PALA was 168 ml/kg.

Urine concentrations were even higher than plasma levels; only 0.1 μl/tube was used. The 27-hour cumulative urinary excretion was 74.5% of the dose (fig 3).

Data on other patients are being collected and will be reported separately.

CONCLUSIONS

The working range of our assay is 1–8 ng of PALA in up to 10 μl of plasma or urine; that is, the sensitivity is 0.1 μg/ml. Since in the current clinical trial the patient receiving the lowest dose of 800 mg/m² showed 71 μg/ml in the first plasma sample and 1620 μg/ml in the urine, and all of the other patients including the patient for whom data are shown (figs 2 and 3) have had higher levels, the assay was readily applicable after a ≥ 3-decade decrease. Clearly, our method is useful for the direct assay of PALA in blood, urine, or cerebrospinal fluid, and we believe it can be adapted to tissues. Since the measurement is of inhibition of the target enzyme, the assay measures PALA and any active metabolites. Inactive metabolites would not be detected. However, no metabolites have yet been reported (9).

REFERENCES

1. SWYRYD EA, SEAVER SS, and STARK GR. *N*-(Phosphonacetyl)-L-aspartate, a potent transition state analog inhibitor of aspartate transcarbamylase, blocks proliferation of mammalian cells in culture. J Biol Chem 249:6945–6950, 1974.
2. HOOGENRAAD NJ. Reaction mechanism of aspartate transcarbamylase from mouse spleen. Arch Biochem Biophys 161:76–82, 1974.
3. JOHNSON RK, INOUYE T, GOLDIN A, ET AL. Antitumor activity of *N*-(phosphonacetyl)-L-aspartic acid, a transition-state inhibitor of aspartate transcarbamylase. Cancer Res 36:2720–2725, 1976.
4. PORTER RW, MODEBE MO, and STARK GR. Aspartate transcarbamylase: kinetic studies of the catalytic subunit. J Biol Chem 244:1846–1859, 1969.
5. MORI M, ISHIDA H, and TATIBANA M. Aggregation states and catalytic properties of the multienzyme complex catalyzing the initial steps of pyrimidine biosynthesis in rat liver. Biochemistry 14:2622–2630, 1975.
6. HOOGENRAAD NJ, LEVINE RL, and KRETCHMER N. Copurification of carbamoyl phosphate synthetase and aspartate transcarbamoylase from mouse spleen. Biochem Biophys Res Commun 44:981–988, 1971.
7. DIXON M, and WEBB EC. Enzymes, 2nd ed. New York, Academic Press, 1964.
8. TATIBANA M, and ITO K. Control of pyrimidine biosynthesis in mammalian tissues. I. Partial purification and characterization of glutamine-utilizing carbamoyl phosphate synthetase of mouse spleen and its tissue distribution. J Biol Chem 244:5403–5413, 1969.
9. CHADWICK M, SILVEIRA DM, MACGREGOR JA, ET AL. Physiological disposition of PALA in several species. Proc Am Assoc Cancer Res and ASCO 19:182, 1978.

Reduction of Daunorubicin Lethality and Myocardial Cellular Alterations by Pretreatment With ICRF-187 in Syrian Golden Hamsters [1,2]

E. Herman, [3,*] B. Ardalan, [4] C. Bier, [5] V. Waravdekar, [6] and S. Krop [3]

Daunorubicin and adriamycin have been utilized against a wide variety of neoplasms. Their therapeutic use has been limited by toxic effects such as bone marrow suppression and cardiomyopathy (1–3). Considerable effort is currently underway to reduce the toxicity of these two agents while maintaining their therapeutic efficacy. In previous studies, a significant reduction in toxicity from high doses of daunorubicin occurred when animals were pretreated with ICRF-159 (4). This protective effect is of great interest, since ICRF-159 also possesses antineoplastic activity (5,6) and, in addition, enhances the antitumor activity of daunorubicin (7).

ICRF-187 [1,2-bis(3,5-dioxopiperazin-1-yl)propane; NSC-169780], the *d*-isomer of ICRF-159 (which is a racemic mixture), has recently been made available for study. This compound has the advantage of being more soluble than ICRF-159. The present studies were initiated to determine whether ICRF-187 also would reduce daunorubicin toxicity as well as prevent cardiac and hepatic cellular damage.

METHODS AND RESULTS

Survival Study

ICRF-187 (10 mg/ml) was dissolved in 0.9% normal physiologic saline prior to use. Twenty-five male Syrian golden hamsters (ARS Sprague-Dawley, Madison, Wis), weighing 70–110 g, each received 100 mg of ICRF-187/kg ip while 45 additional animals received a comparable volume of saline ip. After 1 hour, animals pretreated with ICRF-187 and 25 animals pretreated with saline were anesthetized with pentobarbital sodium (45 mg/g) and administered 25 mg of daunorubicin/kg via the femoral vein. Daunorubicin was dissolved, prior to use, in 0.9% normal physiologic saline so that the dose volume for each animal was approximately 0.3 ml. Twenty saline-pretreated animals served as controls and received an equivalent volume of saline iv.

Only 10% of the saline-pretreated hamsters injected with daunorubicin alone survived for 3 weeks, whereas 80% of the ICRF-187-pretreated animals were alive 5 weeks after administration of daunorubicin (fig 1). During this period the animals pretreated with saline and administered daunorubicin lost an average of 37 g (−36%) of body weight, in contrast to ICRF-187-pretreated animals, which lost an average of 11.4 g (−10%) of body weight ($P < 0.005$). At the end of 5 weeks the 18 surviving ICRF-187-treated animals had gained an average of 4 g above the original control weight. These animals were observed for up to 4 months after daunorubicin administration. After 3 months, 11 of the hamsters survived and by 4 months there were still nine ICRF-187-pretreated animals alive. Control animals gained an average of 28.7 g (+28%) of body weight over the 5-week observation period (fig 2).

The effect of ICRF-187 pretreatment on daunorubicin toxicity was examined in a second animal species. Five adult male Fischer rats, weighing 130–160 g, were pretreated with 100 mg of ICRF-187/kg ip 1 hour prior to a 25-mg infusion of daunorubicin/kg iv. A second group of five rats received only the infusion of daunorubicin at the same dose level. These animals were anesthetized with pentobarbital sodium (45 mg/g) after which daunorubicin was administered through a femoral venous cannula. At the end of the third week, all five rats pretreated with ICRF-187 were alive while only one of the five animals pretreated with saline survived.

Hematologic Study

Hamsters were divided into three groups of four animals and were injected with saline plus dauno-

[1] Received June 29, 1978; revised Oct 6, 1978; accepted Oct 31, 1978.

[2] Daunorubicin and ICRF-187 were kindly supplied by Dr. H. B. Wood, Jr, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[3] Division of Drug Biology, Food and Drug Administration, Washington, DC.

[4] Division of Oncology, Georgetown University School of Medicine, Washington, DC.

[5] Department of Pathology, McGill University, Montreal, Quebec, Canada.

[6] Office of Program Planning and Analysis, Office of the Director, National Cancer Institute, Bethesda, Md.

**Reprint requests to:* Dr. Eugene H. Herman, Division of Drug Biology, HFD-413, Food and Drug Administration, 200 C St, SW, Washington, DC 20204.

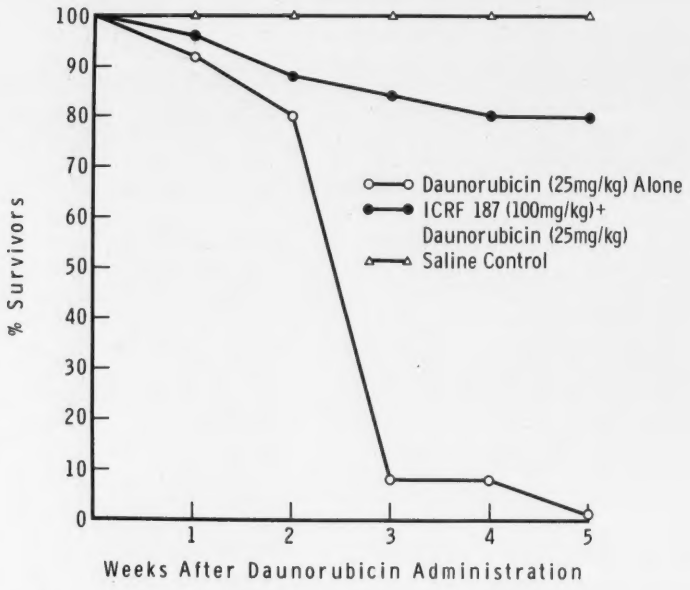


FIGURE 1.—Survival of hamsters after daunorubicin administration with and without ICRF-187 pretreatment.

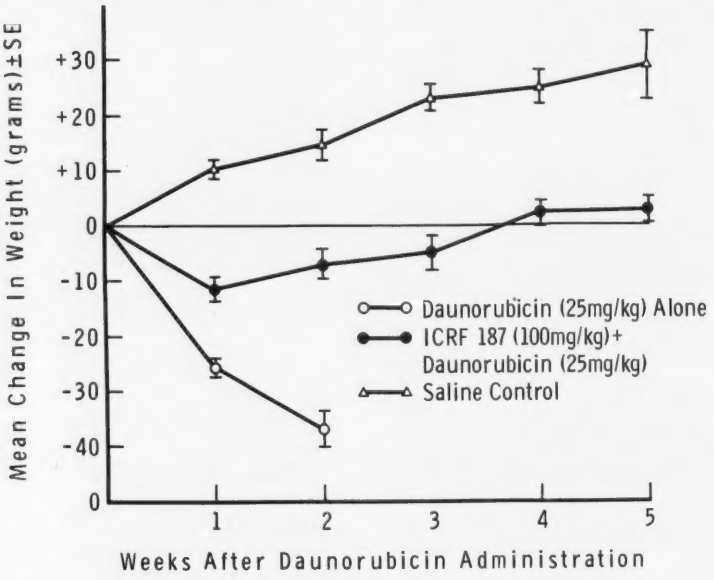


FIGURE 2.—Weight changes in hamsters after daunorubicin administration with and without ICRF-187 pretreatment.

rubicin, daunorubicin plus ICRF-187, and saline alone as previously described. After 72 hours, blood samples were obtained from the retro-orbital sinus of each animal and the total wbc count was determined with the Coulter counter B_{ZI}. At this time, the average wbc count in saline-injected control animals was 5700/μl compared to an average wbc count of 1800/μl in the saline plus daunorubicin-treated hamsters and 800/μl in the combined ICRF-187 plus daunorubicin-treated hamsters ($P < 0.005$).

Histopathologic Study of Heart and Liver Tissues

Forty-four hamsters were injected with 100 mg of ICRF-187/kg ip followed in 1 hour by 25 mg of daunorubicin/kg iv; saline ip followed in 1 hour by 25 mg of daunorubicin/kg iv, or saline ip followed in 1 hour by saline iv. Animals were killed at intervals of 1, 2, 4, 6, and 10 weeks after daunorubicin administration. The hearts and livers were excised and fixed in 10% buffered formalin. Blocks of heart and liver tissues were embedded in paraffin and plastic resin. Sections were prepared and stained with toluidine blue (heart) or hematoxylin and eosin (liver and heart) for light microscopic examination. Light microscopic examination of the heart 1 and 2 weeks after daunorubicin administration demonstrated diffuse vacuolation of myocardial cells from both ICRF-187-pretreated and saline-pretreated animals. At 4 weeks, areas of focal and diffuse vacuolation of cardiac muscle cells were still noted in both groups of animals. However, by 6 weeks, the incidence of vacuolation was diminished in ICRF-187-pretreated hamsters. Vacuolation was rarely seen in the heart sections from ICRF-187-pretreated animals examined 10 weeks after daunorubicin administration. Myocardial alterations were not observed in any of the saline-control animals.

The light microscopic examination of liver tissue obtained at the various intervals after daunorubicin administration in both ICRF-187-pretreated and saline-pretreated animals demonstrated comparable slight vacuolation of the hepatocytes. Although some recovery was noted, vacuolation of hepatocytes was still evident in livers taken from ICRF-187-pretreated animals 10 weeks after daunorubicin treatment.

DISCUSSION

In the present studies, ICRF-187 was shown to exert a significant reduction in the toxic effects of a lethal dose of daunorubicin. Previously, similar results were obtained with the racemic compound, ICRF-159 (3). This reduction in toxicity was initially observed during the first 2 weeks following daunorubicin administration as ICRF-187-pretreated animals lost less weight than saline-pretreated hamsters. ICRF-187 does not interfere with myelosuppression induced by daunorubicin. For example, the decrease in the wbc count after daunorubicin administration was greater when the two drugs were used in combination. At the cellular level, microscopic examination of hearts obtained from ICRF-187-pretreated and saline-pretreated animals killed during the initial 2-week period revealed comparable diffuse vacuolation. The liver alterations were also similar in both groups. The hearts and livers from control animals were devoid of such alterations.

The reduction in daunorubicin toxicity by ICRF-187 was most noticeable after 3 weeks. At this time only 10% of the hamsters treated with daunorubicin alone were alive while 80% of the ICRF-187-pretreated animals survived. The light microscopic examination of the myocardial cells in ICRF-187-pretreated animals after 6 weeks demonstrated a decrease in the incidence of myocardial vacuolation. Furthermore, by 10 weeks, there was essentially no morphologic difference in the structure of the myocardial cells between ICRF-187-pretreated and saline-control animals. In this study, emphasis was on heart and liver tissue, but it appears that the observed myocardial and hepatic changes may not fully account for the ultimate lethality of daunorubicin. The lethal effects of daunorubicin may be related to profound alterations in other tissues such as the gastrointestinal tract. Nonpretreated animals stopped eating and lost almost one third of their total weight by the end of the second week. Under these wasting conditions the animals were unable to sustain themselves. ICRF-187 protection against daunorubicin toxicity in a variety of tissues, such as the kidney and gastrointestinal tract, is currently under investigation.

α-Tocopherol pretreatment has been found to reduce adriamycin toxicity in mice (8). The protective effect is most apparent during the first 3 weeks. Recent studies indicate that the initial α-tocopherol protection is temporary and that by 60 days the death rate in both treated and nontreated mice is the same (9). In contrast, ICRF-187 pretreatment appears to afford both initial and long-term reduction in daunorubicin toxicity, since a significant number of animals survived for > 4 months. This long-term survival occurred even though the dose of daunorubicin was considerably higher than the reported dose of adriamycin used in the α-tocopherol studies in mice. The mechanism by which ICRF-187 reduced daunorubicin toxicity is not clear.

ICRF-159, the racemic compound, has been found

to exert several diverse effects. It prevents the metastasis of implanted tumor cells in animals by normalizing the vasculature of developing tumors (10). There is evidence, both experimental and clinical, that ICRF-159 enhances the effect of radiation on tumor cells (11). Presumably, ICRF-187 exerts a similar effect. It seems unlikely that either of these actions would lead to a reduction in daunorubicin toxicity. Since ICRF-187 is a non-polar derivative of EDTA, its presence might interfere with certain toxic manifestations of daunorubicin by altering critical cation concentrations. These questions concerning ICRF-187 are currently under investigation.

REFERENCES

1. TAN C, TASAKA H, YU KP, ET AL. Daunomycin, an antitumor antibiotic, in the treatment of neoplastic disease. Clinical evaluation with special reference to childhood leukemia. Cancer 20:333–353, 1967.
2. SERPICK AA, and HENDERSON ES. Observations on toxicity and clinical trials with daunomycin. Pathol Biol 15:955–957, 1967.
3. MARAL R, BOUFAT G, DUCROT R, ET AL. Etude toxicologique et activité antitumorale expérimentale de la rubidomycine (13.057 R.P.). Pathol Biol 15:903–908, 1967.
4. HERMAN EH, MHATRE RM, and CHADWICK DP. Modification of some of the toxic effects of daunomycin (NSC-82,151) by pretreatment with the antineoplastic agent ICRF 159 (NSC-129,943). Toxicol Appl Pharmacol 27:517–526, 1974.
5. HELLMANN K. ICRF 159 and other bisdioxopiperazines. *In* Chemotherapy. New York, Plenum Press, 1976, vol 7, pp 183–186.
6. CREIGHTON AM, HELLMANN K, and WHITECROSS S. Antitumour activity in a series of bisdiketopiperazines. Nature 222:384–385, 1969.
7. WOODMAN RJ, CYSYK RL, KLINE I, ET AL. Enhancement of the effectiveness of daunorubicin (NSC-82151) or adriamycin (NSC-123127) against early mouse L1210 leukemia with ICRF-159 (NSC-129943). Cancer Chemother Rep 59:689–695, 1975.
8. MYERS CE, MCGUIRE WP, LISS RH, ET AL. Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. Science 197:165–168, 1977.
9. GRAM TE, MIMNAUGH B, SIKIC BI, ET AL. Studies on the interaction between adriamycin and α-tocopherol in vivo. *In* Proceedings of the 7th International Congress of Pharmacology, 1978, p 350.
10. LE SERVE AW, and HELLMANN K. Metastases and the normalization of tumour blood vessels by ICRF 159: a new type of drug action. Br Med J 1:597–601, 1972.
11. BAKOWSKI MT. ICRF 159, (±)1,2-di(3,5-dioxopiperazin-1-yl)-propane NSC-129,943; Razoxane. Cancer Treat Rev 3:95–107, 1976.

Mechanism of the Antimetastatic Action of Dimethyltriazenes [1,2]

G. Sava, T. Giraldi,* L. Lassiani, and C. Nisi [3,4]

SUMMARY

Three aryldiazonium cations, D-NO_2, D-$CONH_2$, and D-CH_3, have been examined as possible metabolites responsible for the selective antimetastatic effects reported for three aryldimethyltriazenes, DM-NO_2, DM-$CONH_2$, and DM-CH_3. When tested on the formation of spontaneous lung metastases in mice bearing Lewis lung carcinoma, they caused no significant reduction either in the number or the weight of pulmonary metastases. DM-COOK, another metabolite of the active DM-$CONH_2$, proved, on the contrary, to be highly effective. When administered as an aqueous solution, a marked inhibition of the number and weight of lung nodules was observed at the three dose levels used. A large proportion of animals were also found to be free of large metastases, and a smaller number were completely free of macroscopically detectable lung colonies. The activity of DM-COOK and DM-COOH, when administered as suspensions in oil, was significantly less pronounced. DM-CH_3, DM-NO_2, and DM-COOK were devoid of effects on the formation of artificial metastases obtained by iv injection of tumor cells. This finding indicates that the inhibitory effects of these compounds on the formation of spontaneous pulmonary metastases are not due to inhibition of replication of tumor cells localized in the lungs.

[Cancer Treat Rep 63:93–98, 1979]

DTIC has activity against several rodent transplantable tumors (1), and is also useful in treating human malignancies (2–4). Recently, DTIC, an analog (4-carbethoxy-5-[3,3-dimethyl-1-triazeno]-2-methylimidazole; BRL-51308), and a benzenoid dimethyltriazene (DM-$CONH_2$) have been shown to possess selective antimetastatic activity in mice bearing Lewis lung carcinoma (3LL) (5,6). The antimetastatic effects consisted of a marked depression of the formation of lung metastases, while no parallel inhibition of the growth of the subcutaneous primary tumor was observed. The fact that the inhibition of metastasis formation was not due to cytotoxic effects of the tested triazenes for tumor cells localized in the lungs was also indicated by the absence of any significant inhibition of the fractional incorporation of ^{3}H-thymidine into the DNA of tumor cells (5).

It has been postulated that dimethyltriazenes require metabolic activation to exhibit their cytotoxic (7,8), mutagenic (9,10), teratogenic, and carcinogenic (11) effects, and it may be that this is also required for their selective antimetastatic properties. Two different pathways have been proposed as producing possible active metabolites for dimethyltriazenes. The first consists of the generation, by oxidative microsomal *N*-demethylation, of monomethyltriazenes (7). The second is the proton-catalyzed hydrolysis of dimethyltriazenes to aryldiazonium cations (12).

Consequently, two other benzenoid triazenes, DM-NO_2 and DM-CH_3, were evaluated for their differential effects on primary tumor growth and lung metastasis formation, in comparison with DM-$CONH_2$; the three corresponding monomethyltri-

[1]Received July 21, 1978; revised Nov 13, 1978; accepted Nov 20, 1978.

[2]Supported by grants CT 77.01110.03 and CT 77.01370.04 from the Consiglio Nazionale delle Ricerche and by a grant from the Università di Trieste.

[3]Istituto di Farmacologia (*G. Sava* and *T. Giraldi*) and Istituto di Chimica Farmaceutica (*L. Lassiani* and *C. Nisi*), Università di Trieste, Italy.

[4]The skillful technical assistance of G. Fonzari is gratefully acknowledged.

**Reprint requests to:* Dr. Tullio Giraldi, Istituto di Farmacologia, Università di Trieste, Via A. Valerio N. 32, 34100 Trieste, Italy.

azenes were also examined. All three dimethyltriazenes markedly and similarly depressed the formation of spontaneous lung secondaries. On the other hand, the most stable compound for hydrolysis to aryldiazonium cations (DM-NO_2, half-life = 160 days) significantly inhibited the growth of the subcutaneous tumor, whereas the short-lived DM-CH_3 (half-life = 32 minutes) was completely inactive. The effects caused by the monomethyltriazenes were less pronounced than those of the corresponding dimethyltriazenes.[5] This indicated a marginal role for microsomal oxidative *N*-demethylation as the metabolic pathway responsible for the generation of the active species. On the contrary, DM-COOK, examined as another metabolite of DM-$CONH_2$, proved to be highly active.[5]

The purpose of this investigation was, therefore, to examine in more detail the effects of 1-*p*-(3,3-dimethyl-1-triazeno)benzoic acid (DM-COOH) and its potassium salt (DM-COOK) on the formation of spontaneous metastases. In order to examine the relevance of the hydrolysis of dimethyltriazenes to aryldiazonium cations for their antimetastatic effects, the activity of aryldiazonium fluoroborates on primary tumor growth and spontaneous lung metastasis formation was also examined. Furthermore, the effects of dimethyltriazenes on the formation of artificial metastases, obtained by iv injection of tumor cells, have been investigated.

MATERIALS AND METHODS

Synthesis.—The synthesis of the compounds used in this investigation was performed following previously reported procedures (table 1, refs 13–18).

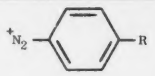
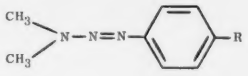
In vivo experimental system.—The 3LL line used was kindly provided by Mr. I. Wodinsky, Arthur D. Little Inc (Cambridge, Mass). For studying the formation of spontaneous metastases, the tumor was transplanted in C57BL mice by aseptically implanting sc in the axillary region 10^6 viable cells of a single cell suspension which was prepared as described by Ovejera et al (19). Artificial metastases were obtained by iv injection of 2.5×10^5 tumor cells. All of the compounds were administered ip in volumes of 0.05 ml/10 g of animal weight as a suspension in olive oil; DM-COOK was also used as a solution in water containing 0.1 M $NaHCO_3$.

Evaluation of tumor growth and metastasis formation.—Primary tumor weight was determined at the end of treatment by caliper measurements, assuming tumor density to be equal to 1, as the volume of the rotation ellipsoid with the long and short axes equal to a and b respectively:

$$\text{tumor weight} = \pi/6 \times a^2 \times b. \quad [1]$$

The number of lung metastases was determined when the mice were killed using a dissection magnifying lens. The mass of metastases was estimated as the sum of their individual weights determined using equation [1].

TABLE 1.—Chemical structures and abbreviations of the tested compounds

R =		Abbreviation	Ref No.
			
$-NO_2$	1-*p*-Nitrophenyldiazonium fluoroborate salt	D-NO_2	13
$-CONH_2$	1-*p*-Carboxamidophenyldiazonium fluoroborate	D-$CONH_2$	14
$-CH_3$	1-*p*-Methylphenyldiazonium fluoroborate salt	D-CH_3	15
			
$-NO_2$	1-*p*-Nitrophenyl-3,3-dimethyltriazene	DM-NO_2	16
$-CH_3$	1-*p*-Tolyl-3,3-dimethyltriazene	DM-CH_3	17
-COOH	*p*-(3,3-Dimethyl-1-triazeno)benzoic acid	DM-COOH	18
-COOK	*p*-(3,3-Dimethyl-1-triazeno)benzoic acid potassium salt	DM-COOK	18

RESULTS

The highest doses used for each compound examined are equitoxic and equal to one half of the LD50 determined by the method of Litchfield and Wilcoxon (20) in normal mice, using the treatment schedule and vehicle identical to those used in the following experiments.

Effects on Spontaneous Metastases

The effects obtained from testing aryldiazonium salts on primary tumor growth and spontaneous lung metastasis formation are reported in table 2. No significant effect was observed for any of the compounds either on the number or the weight of lung metastases. D-NO_2 and D-CH_3 significantly

[5]Giraldi T, Guarino AM, Nisi C, et al. Selective antitumor and antimetastatic effects of benzenoid triazenes in mice bearing Lewis lung carcinoma. Submitted to *Cancer Research*.

TABLE 2.—Antitumor and antimetastatic effects of *p*-substituted phenyldiazonium fluoroborate salts*

Compound	Dose (mg/kg/day)	Primary tumor weight	Effects on metastases			
			No.			
			Small‡	Large	Total	Weight
Controls†	—	100 ± 12 (a)	100 ± 8 (b)	100 ± 20 (c)	100 ± 8 (d)	100 ± 16 (e)
D-CH_3	19	47 ± 5§	107 ± 10	137 ± 16	113 ± 8	144 ± 24
	9.5	72 ± 12	91 ± 12	100 ± 21	93 ± 9	116 ± 21
	4.75	75 ± 14	99 ± 12	170 ± 30	112 ± 14	206 ± 43
D-$CONH_2$	30	75 ± 7	81 ± 12	90 ± 21	98 ± 11	111 ± 28
	15	73 ± 11	100 ± 7	102 ± 19	100 ± 8	189 ± 35
	7.5	86 ± 12	95 ± 13	121 ± 23	100 ± 14	133 ± 23
D-NO_2	19	59 ± 8§	146 ± 18	120 ± 23	138 ± 14	130 ± 21
	9.5	57 ± 6§	113 ± 10	121 ± 11	114 ± 8	161 ± 25
	4.75	79 ± 8	91 ± 7	106 ± 17	94 ± 6	167 ± 28

*Groups of 8 mice were treated daily ip from Day 1 to Day 14 after tumor transplantation. Primary tumor weight was evaluated on Day 15, and the examination of lungs for metastases was performed when mice were killed on Day 21. Each value is expressed as % ratio of the average (± SE) for each treated group to that of controls. Statistical analysis performed is the Student Neumann-Keul test (ref 21).

†Actual value found in the control group: (a) = 1.3 ± 0.16 g; (b) = 27 ± 2.0; (c) = 6.1 ± 1.2; (d) = 33 ± 2.7; (e) = 130 ± 21 mg.

‡Diameter < 2 mm.

§Significantly different from controls ($P < 0.05$).

reduced the growth of the subcutaneous tumor at their highest doses.

The results obtained when examining DM-COOH and its potassium salt (DM-COOK) are reported in table 3. DM-COOH was devoid of any inhibitory effect on the growth of the subcutaneous implant. The effects of DM-COOH on metastases consisted of a significant reduction in the number of small metastases and in their total number at the three dose levels tested. DM-COOK significantly reduced the growth of the primary tumor at the highest dose used, given either as an aqueous solution or as an oil suspension. A marked inhibition of lung metastasis formation was evident only at the highest dose level when DM-COOK was given suspended in oil. However, a pronounced inhibition was caused by all of the three dose levels tested for DM-COOK administered in water; a large proportion of animals were found free of large metastases and a smaller number were completely free of macroscopically detectable lung colonies. This response was found to be dose-dependent.

Effects on Artificial Metastases

The effects obtained in mice treated daily for 8 days following iv injection of tumor cells, with doses of dimethyltriazenes equitoxic and effective against the formation of spontaneous lung metastases, are reported in table 4. No significant difference in the formation of lung colonies, as compared with controls, was caused by any of the triazenes.

DISCUSSION

Imidazoles and aryl-3,3-dimethyltriazenes have been shown to possess selective antimetastatic properties in mice bearing 3LL (5).[6] Of the two different pathways proposed as producing possible active metabolites for dimethyltriazenes, the oxidative microsomal *N*-demethylation to the corresponding monomethyltriazenes (7) and the proton-catalyzed hydrolysis to the aryldiazonium cations (12), the former appears to be marginal, since the effects in mice bearing 3LL caused by monomethyltriazenes are less pronounced than those caused by the parent dimethyl derivatives.[6] With respect to the second metabolic pathway, a correlation appeared between half-life of hydrolysis to aryldiazonium cations and effects in mice bearing 3LL. In fact, the long-lived DM-NO_2 (half-life = 160 days) depressed primary tumor growth and metastasis formation, whereas the short-lived DM-CH_3 (half-life = 32 minutes) inhibited metastasis formation while causing no effect on primary tumor growth.[6] It would thus appear that the antimetastatic effects of DM-NO_2 might be attributed to cytotoxic effects which are evident in the primary tumor, whereas DM-CH_3 inhibits the formation of lung secondaries by selective antimetastatic effects. Examination of the effects obtained by systemic administration of aryldiazonium fluoroborates indicates that their properties are markedly different from those of the corresponding dimethyltriazenes. None of the diazonium salts were capable of significantly inhibiting the formation of lung metastases. Furthermore, some

[6]*See* footnote 5.

TABLE 3.—Antitumor and antimetastatic effects of *p*-(3,3-dimethyl-1-triazeno)benzoic acid and its potassium salt*

Compound	Dose (mg/kg/day)	Vehicle	Primary tumor weight	Effects on metastases				Animals free of large metastases	Animals free of metastases
				No.			Weight		
				Small‡	Large	Total			
Controls†	—	Olive oil	100 ± 10 (a)	100 ± 13 (b)	100 ± 13 (c)	100 ± 13 (d)	100 ± 16 (e)	2/13	0/13
DM-COOH	25	Olive oil	119 ± 13	51 ± 10¶	45 ± 21	49 ± 11§	41 ± 16	2/8	0/8
	12.5	Olive oil	109 ± 8	49 ± 6¶	95 ± 22	57 ± 8§	83 ± 23	1/8	0/8
	6.25	Olive oil	114 ± 14	46 ± 14¶	68 ± 16	48 ± 15§	53 ± 19	2/8	0/8
DM-COOK	50	Olive oil	70 ± 10§	23 ± 5¶	24 ± 5§	24 ± 5¶	19 ± 6§	2/8	1/8
	25	Olive oil	79 ± 11	46 ± 13¶	53 ± 15	49 ± 14§	64 ± 28	0/8	0/8
Controls†	—	0.1 N $NaHCO_3$	100 ± 19 (f)	100 ± 13 (g)	100 ± 14 (h)	100 ± 13 (i)	100 ± 17 (j)	1/15	0/15
DM-COOK	50	0.1 N $NaHCO_3$	59 ± 6§	19 ± 6¶	31 ± 18§	16 ± 6§	8 ± 5¶	8/10	2/10
	25	0.1 N $NaHCO_3$	78 ± 9	22 ± 7¶	16 ± 4§	16 ± 6§	8 ± 3¶	5/8	1/8
	12.5	0.1 N $NaHCO_3$	73 ± 6	26 ± 4¶	20 ± 5§	23 ± 3§	11 ± 3¶	2/8	0/8

*Groups of 8–15 mice were treated daily ip from Day 1 to Day 14 after tumor transplantation. Primary tumor weight was evaluated on Day 15, and the examination of lungs for metastases was performed when mice were killed on Day 21. Each value is expressed as % ratio of the average (± SE) for each treated group to that of controls. Statistical analysis performed is the Student Neumann-Keul test (ref 21).

†Actual value found in the control group: (a) = 1.2 ± 0.10 g; (b) = 26 ± 3.4; (c) = 6.2 ± 0.8; (d) = 31 ± 4.1; (e) = 65 ± 10 mg; (f) = 1.9 ± 0.37 g; (g) = 25 ± 3.4; (h) = 8.1 ± 1.1; (i) = 33 ± 4.3; (j) = 100 ± 17 mg.

‡Diameter < 2 mm.

§Significantly different from controls ($P < 0.05$).

¶Significantly different from controls ($P < 0.01$).

TABLE 4.—Effects of *p*-substituted aryldimethyltriazenes on artificial metastases*

Compound	Dose (mg/kg/day)	Effect on metastases			
		No.			
		Small‡	Large	Total	Weight
Controls†	—	100 ± 16.2 (a)	100 ± 20.0 (b)	100 ± 11.3 (c)	100 ± 24.1 (d)
DM-CH_3	25	124.3 ± 29.7	135.0 ± 38.3	120.6 ± 28.6	143.2 ± 49.7
DM-NO_2	100	137.8 ± 59.4	100.0 ± 35.0	108.2 ± 44.3	100.4 ± 24.3
DM-COOK	50	113.5 ± 24.3	86.7 ± 13.3	99.0 ± 27.8	106.1 ± 22.2

*Groups of 10 mice were treated daily ip from Day 1 to Day 8 after iv injection of 2.5×10^5 tumor cells; the examination of lungs for metastases was performed when mice were killed on Day 15. Each value is expressed as % ratio of the average (± SE) for the treated group to that of controls. Statistical analysis performed is the Student Neumann-Keul test (ref 21); none of the values is significantly different from controls.

†Actual value found in the control group: (a) = 3.7 ± 0.6; (b) = 6.0 ± 1.2; (c) = 9.7 ± 1.1; (d) = 75 ± 18 mg.

‡Diameter < 2 mm.

degree of inhibition of primary tumor growth was observed, the most effective compound being D-CH_3. This is in marked contrast with the activity of dimethyltriazenes, since DM-CH_3 was completely inactive in the subcutaneous implant.[7] This indicates that the effects of DM-CH_3, the only triazene which can generate appreciable amounts of diazonium cations in vivo, cannot be reproduced by the systemic administration of its corresponding diazonium salt, which implies that this species does not participate in the antimetastatic activity of DM-CH_3 or that localized generation of diazonium ions is required. Indeed, the two species have widely different properties: DM-CH_3 is nonpolar and liposoluble, while D-CH_3 is an ionic species. These two compounds may consequently have sharply different pharmacokinetic properties and thus different concentrations at various sites after in vivo administration.

Independent of their possible metabolic role for the activity of DM-$CONH_2$, paracarboxyphenyltriazenes appear to have interesting activity. DM-COOK, particularly when given as an aqueous solution, has remarkable antimetastatic activity over a wide range of doses. Its water solubility is an obvious advantage, in terms of ease of administration, over the other triazenes.

Considering the mechanism of the antimetastatic action of dimethyltriazenes, it is worth noting the contrast between their marked inhibitory effects on spontaneous metastasis formation and their lack of effect on the formation of artificial metastases obtained by iv injection of tumor cells. This finding is consistent with the observed absence of cytotoxicity for pulmonary and subcutaneous 3LL tumors found for DTIC, BRL-51308, and DM-$CONH_2$ when examining the fractional incorporation of ^{3}H-thymidine into the DNA of tumor cells (5). An examination of the effectiveness of the use of dimethyltriazenes as prophylactic adjuvants to the surgical treatment of animal tumors is in progress, and preliminary results, obtained with DM-COOK, are promising. Also in progress is a study of the biochemical mechanism of the antimetastatic properties of dimethyltriazenes.

[7] *See* footnote 5.

REFERENCES

1. VENDITTI JM. Antitumor activity of DTIC (NSC-45388) in animals. Cancer Treat Rep 60:135–140, 1976.
2. BERETTA G, BONADONNA G, BAJETTA E, ET AL. Combination chemotherapy with DTIC (NSC-45388) in advanced malignant melanoma, soft tissue sarcomas, and Hodgkin's diseases. Cancer Treat Rep 60:205–211, 1976.
3. COMIS RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
4. SLAVIK M. Clinical studies with DTIC (NSC-45388) in various malignancies. Cancer Treat Rep 60:213–214, 1976.
5. GIRALDI T, HOUGHTON PJ, TAYLOR DM, ET AL. Antimetastatic action of some triazene derivatives against the Lewis lung carcinoma in mice. Cancer Treat Rep 62:721–725, 1978.
6. HEYES J. Antimetastatic effect of 4-carbethoxy-5-(3,3-dimethyl-1-triazeno)-2-methylimidazole. J Natl Cancer Inst 53:279–280, 1974.
7. CONNORS TA, GODDARD PM, MERAI K, ET AL. Tumor inhibitory triazenes: structural requirements for an active metabolite. Biochem Pharmacol 25:241–246, 1976.
8. ABEL G, CONNORS TA, and GIRALDI T. In vitro metabolic activation of 1-*p*-carboxamidophenyl-3,3-dimethyltriazene to cytotoxic products. Cancer Lett 3:259–264, 1977.
9. KOLAR GF, FAHRIG R, and VOGEL E. Structure-activity dependence in some novel ring-substituted 3,3-dimethyl-1-phenyltriazenes. Genetic effects in *Drosophila melanogaster* and in *Saccharomyces cerevisiae* by a direct and a host-mediated assay. Chem Biol Interact 9:365–378, 1974.
10. MALAVEILLE C, KOLAR GF, and BARTSCH H. Rats and mouse tissue-mediated mutagenicity in *Salmonella typhimurium*. Mutat Res 36:1–10, 1976.

11. DRUCKREY H. Specific carcinogenic and teratogenic effects of "indirect" alkylating methyl and ethyl compounds, and their dependency on stages of ontogenic developments. Xenobiotica 3:271–303, 1973.
12. KOLAR GF, and PREUSSMANN R. Validity of a linear Hammet plot for the stability of some carcinogenic 1-aryl-3,3-dimethyltriazenes in an aqueous system. Z Naturforsch [B] 26:950–953, 1971.
13. CURTIN DY, and TVETEN JL. Reaction of diarylzine reagents with aryldiazonium salts. Direct formation of *cis*-azo compounds. J Org Chem 26:1764–1768, 1961.
14. GIRALDI T, NISI C, and SAVA G. Investigation on the oxidative *N*-demethylation of aryl triazenes in vitro. Biochem Pharmacol 24:1793–1797, 1975.
15. BALZ G, and SCHIEMANN G. Uber aromatische fluorverbindungen. I. Ein neues verfahren zu ihrer darstellung. Chem Ber 60B:1186–1190, 1927.
16. ELKS J, and HEYD DH. Union of aryl nuclei. Part VI. Reactions with 1-aryl-3,3-dimethyltriazenes. J Chem Soc, 1943, pp 441–445.
17. KLAGES F, and MESCH W. Addition reactions of triazenes, illustrating the reactivity of N=N double bonds. Chem Ber 88:388–396, 1955.
18. KOLAR GF. Synthesis of biologically active triazenes from isolable diazonium salts. Z Naturforsch [B] 27:1183–1185, 1972.
19. OVEJERA AA, JOHNSON RK, and GOLDIN A. Growth characteristics and chemotherapeutic response of intravenously implanted Lewis lung carcinoma. Cancer Chemother Rep Part 2, vol 5(1):111–125, 1975.
20. LITCHFIELD J, and WILCOXON F. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99–113, 1949.
21. SOKAL RR, and ROHLF FJ. Single classification analysis of variance. *In* Biometry. San Francisco, W. H. Freeman, 1969, pp 204–252.

Comparison of Various Forms of Therapy in Two Different Mouse Tumor Systems [1,2]

Hub F. J. Dullens, [3,*] Claus Vennegoor, [4] Roel A. De Weger, [3] Felice Woutersen, [3] Ruud A. Woutersen, [3] and Willem Den Otter [3]

SUMMARY

At our institute, research is mainly focused on the use of macrophages and lymphocytes for the development of immunotherapy. However, it would not be wise to continue the development of a cellular therapy if other forms of therapy appeared to be far more effective. In addition, it is not known whether the best therapy for one tumor is also the best therapy for another tumor. For these reasons, we have studied the efficacy of various forms of therapy in two mouse tumor systems which we regard as rather different, ie, the DBA/2-SL2 lymphosarcoma system and the BALB/c-Harding-Passey melanoma (HPM) system. SL2 is a tumor with a high growth rate which grows ip as an ascitic tumor; HPM is a tumor with a low growth rate which grows ip as a solid tumor. In this paper we describe the antitumor effects of various therapies in the two mouse tumor systems. These therapies are: (*a*) hyperimmune peritoneal exudate cells; (*b*) hyperimmune spleen cells; (*c*) immunoglobulin-coated hyperimmune peritoneal exudate cells; (*d*) chlorambucil-antibody complex; (*e*) bacillus Calmette-Guérin; and (*f*) DTIC. The results show that (*a*) HPM is more susceptible to each therapy than SL2 lymphosarcoma, and (*b*) therapy with both hyperimmune as well as immune peritoneal exudate cells is very potent in both systems, eradicating up to 2×10^7 SL2 cells and 10^7 HPM cells (experiments with $> 10^7$ HPM cells were not feasible).

[Cancer Treat Rep 63:99–109, 1979]

The aim of all forms of cancer therapy is to remove or to destroy the tumor without seriously damaging the host. Often, this can be achieved by surgical excision or by radiotherapy. Another possible approach is the use of chemotherapeutics. The fourth approach is immunotherapy. The numerous attempts to apply immunologic methods to the treatment of cancer in experimental systems as well as in man have been reviewed (1–4). The potential methods of tumor immunotherapy can be divided into three main groups: (*a*) active immunotherapy (immunization with tumor cells or extracts, or nonspecific stimulation of the immune response by adjuvants); (*b*) passive immunotherapy (transfer of antitumor sera); and (*c*) adoptive immunotherapy (transfer of immune or nonimmune lymphoid cells). There are promising developments in each of these groups. (*a*) Nonspecific stimulation of host resistance to tumors, in particular, the use of bacillus Calmette-Guérin (BCG) (5–7), *Corynebacterium parvum* (8–10), and pyran-copolymer (11,12), has aroused considerable interest. (*b*) Passive immunotherapy has been abandoned almost completely because success is doubtful and many investigators are afraid of enhanced tumor growth (1). However, the use of antitumor antibodies as carriers for various cytotoxic drugs, eg, radionuclides (13), methotrexate (14), antidiphtheria toxin (15), and especially chlorambucil (16–18), has been described. (*c*) Adoptive immunotherapy, the cellular involvement

[1]Received Aug 1, 1978; revised Oct 30, 1978; accepted Nov 6, 1978.

[2]Supported by the Koningin Wilhelmina Fonds and the Utrecht State University.

[3]Department of Pathology, Utrecht State University, The Netherlands.

[4]The Netherlands Cancer Institute, Amsterdam.

**Reprint requests to:* Dr. Hub F. J. Dullens, Department of Pathology, Utrecht State University, Pasteurstraat 2, 3511 HX Utrecht, The Netherlands.

at the site of tumor graft rejection, has been studied extensively. The involvement of macrophages (19–21) and lymphocytes (22–24) is now well-established.

At our institute, research is mainly focused on the use of macrophages and lymphocytes (25–27) for the development of immunotherapy; this includes study of the tumor rejection mechanism to determine the optimal conditions that are to be used. However, it would not be wise to continue the development of a cellular therapy if other forms of therapy appeared to be far more effective. In addition, it is not known whether the best therapy for one tumor is also the best therapy for another tumor. For these reasons, we have studied the efficacy of various forms of therapy in two mouse tumor systems which were chosen because they are rather different, ie, the DBA/2-SL2 lymphosarcoma system and the BALB/c-Harding-Passey melanoma (HPM) system.

SL2 is a tumor with a high growth rate which grows ip as an ascitic tumor. HPM is a tumor with a low growth rate which grows ip as a solid tumor.

We are aware that selection of the two different systems does not allow generalization of the presented results to all other tumor systems.

MATERIALS AND METHODS

Mice

BALB/c, C57BL, and DBA/2 mice (males and females, 6–15 weeks old) were used. The mice were purchased from the Jackson Laboratories, Bar Harbor, Me; Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands; and the Radiobiological Institute TNO, Rijswijk, The Netherlands.

Tumors

Harding-Passey Melanoma

The HPM was found on the left ear of a brown stock mouse in the laboratory of Dr. R. A. Passey of the Leeds University Medical School (England, 1925). More data about the HPM have been published by Stewart et al (28). The tumor grows ip as a solid tumor and was maintained by biweekly ip passage of 10^6 cells. HPM cell suspensions were prepared by the following method: (*a*) the tumor was cut and pressed through gauze, (*b*) the cells were suspended, (*c*) clumps of cells were allowed to settle, (*d*) the supernatant containing free-floating cells was decanted, (*e*) the cell suspension was centrifuged, and (*f*) the cells were resuspended in Fischer's medium (29) and used in the in vivo experiments.

For the fluorescence and cytotoxicity tests the free-floating cells were first cultured in Fischer's medium (supplemented with 10% fetal bovine serum) at 37°C. After 2 days the monolayer of cells was washed with medium. The cells were detached from the bottom by incubation with 0.54 mM EDTA for 10 minutes at 37°C. The cells were washed two times with medium before use.

SL2 Lymphosarcoma

The SL2 lymphosarcoma arose spontaneously as an ascitic tumor in a DBA/2 mouse (Sutton, England, 1969).

TLX9 Thymoma

The TLX9 thymoma was induced by X-irradiation (Sutton, England, 1968) in a C57BL mouse.

P815 Mastocytoma

The P815 mastocytoma is syngeneic to DBA/2 mice.

L5178Y Lymphoma

The L5178Y lymphoma is syngeneic to DBA/2 mice.

These tumors grow ip as ascitic tumors and were maintained by weekly ip passage of 10^6 cells.

Immunization

Immune peritoneal exudate cells (PECs) (and/or spleen cells) were obtained from C57BL mice 10 days after ip injection of 5×10^6 HPM or 10^7 SL2 tumor cells. Hyperimmune PECs (and/or spleen cells) were obtained from C57BL mice that received four ip injections with 5×10^6 HPM or 10^7 SL2 tumor cells given at 9-day intervals. The hyperimmune cells were collected 5 days after the last immunization. We define hyperimmune PECs as cells collected from the peritoneal cavity after hyperimmunization.

Collection of Peritoneal and Spleen Cells

PECs were collected by injecting 5 ml of Fischer's medium into the peritoneal cavity; the cell suspension was withdrawn with a syringe after gentle massaging of the abdomen. Spleen cell suspensions were obtained by perfusion of the spleen with Fischer's medium. The cells were pooled, centrifuged, and resuspended in Fischer's medium. A PEC suspension obtained from C57BL mice contained approximately equal numbers of macrophages and lymphocytes. Usually, some mast cells, polymorphonuclear cells, and plasma cells are present. Spleen cell suspensions contained approximately 10%–15% macrophages.

Purification of Macrophage and Lymphocyte Suspensions

Macrophages were separated from lymphocytes by injecting 0.3 ml of antilymphocyte serum (ALS) (horse-anti mouse) ip into C57BL mice 45 minutes before collection of the PECs. The macrophages were contaminated with < 8% lymphocytes. The lymphocytes were freed of macrophages by exposing the cells to glass (two times for 30 minutes at 37°C). The purified lymphocytes were contaminated with < 7% macrophages.

Antilymphocyte Serum

ALS was donated by Dr. Van Bekkum of the Radiobiological Institute TNO, Rijswijk, The Netherlands. Mouse thymus and lymph node cells (2×10^9) in Freund's complete adjuvant (FCA) were injected into a horse sc, and on Days 21, 22, and 23 further injections of 10^9 mixed lymphoid cells were given iv. On Days 31, 35, and 39, blood was collected and the serum was pooled, heat inactivated, and sterilized by filtration. The ALS was absorbed with C57BL liver homogenate 1:1 (vol/vol) at 4°C for 30 minutes and on C57BL macrophage monolayers exposing the serum for 5 minutes to each of 12 monolayers. The absorbed ALS had no antimacrophage activity in the presence of complement. The cytotoxic titer of this horse-anti-mouse lymphocyte serum was 1:1024 versus thymocytes and 1:64 versus lymph node cells.

Antisera (General Information)

All antisera were produced by the same method. Rabbits or goats received two im injections of 10^7 tumor cells suspended in 1 ml of Fischer's medium mixed with an equal volume of FCA with an interval of 2 weeks, followed 1 and 2 weeks later by an im injection of 10^7 tumor cells without FCA. The sera were collected 7 days after the last immunization and decomplemented at 56°C for 30 minutes. The sera (including the control sera) were adsorbed twice with equal volumes of mouse tissue homogenate (liver, heart, spleen, and kidney) at 4°C–10°C for 30 minutes.

The globulin fractions of the adsorbed antisera were isolated by three successive precipitations with a saturated $(NH4)_2SO_4$ solution (final concentration, 33.3%). This was followed by dialysis against cold 0.01 M phosphate-buffered saline (PBS, pH 7.2). Globulins were freeze-dried and stored at −20°C. The proteins were reconstituted to PBS before use. Immunoelectrophoresis of the isolated globulins showed that the fraction mainly consisted of IgG. Antibody activity of the antisera was shown by:

(*a*) *membrane immunofluorescence*—the indirect method with rabbit-anti-goat-fluorescein-isothiocyanate (RAG-FITC; Nordic Pharmaceuticals and Diagnostics, Tilburg, The Netherlands) or goat-anti-rabbit-FITC (GAR-FITC; Nordic Pharmaceuticals and Diagnostics) was used; and

(*b*) *cytotoxicity tests*—4×10^5 spleen, lymph node, or tumor cells suspended in 1 ml of medium were incubated in Nunc Petri dishes (3 cm in diameter) with 1 ml of reconstituted immunoglobulin (0.2 mg in PBS), and 0.5 ml of guinea pig complement (diluted 1:10) at 37°C for 1 hour. Surviving tumor cells were counted in a hemocytometer using the trypan-blue exclusion test.

Antisera (Specific Information)

Goat-Anti-SL2

Goat-anti-SL2 serum was adsorbed with C57BL tissue homogenates. The immunoglobulin fraction was used for the coating of C57BL immune and hyperimmune PECs. Immunofluorescence tests showed that the SL2 cells were labeled with the goat-anti-SL2 immunoglobulin (0.2 mg/ml). C57BL-derived TLX9 tumor cells did not show any fluorescence with the anti-SL2 immunoglobulin. Incubation of SL2 cells with (*a*) control immunoglobulin of a normal goat plus RAG-FITC or (*b*) RAG-FITC only did not result in significant labeling of the tumor cells.

The cytotoxicity tests showed that 0.2 mg (isolated) anti-SL2 immunoglobulin caused lysis of 100% of the lymphoma cells in the presence of complement within 1 hour.

Rabbit-Anti-SL2

This serum was absorbed with DBA/2 tissue homogenates. The immunoglobulin fraction was used for complexing with chlorambucil. Immunofluorescence tests showed that the SL2 cells were labeled with the rabbit-anti-SL2 serum (titer, 1/240). The antiserum showed some cross-reaction with DBA/2 skin cells, DBA/2-derived P815 cells, and L5178Y cells (titer, 1/60). Incubation of SL2 cells with (*a*) control immunoglobulin of a normal rabbit plus GAR-FITC or (*b*) GAR-FITC only did not result in significant labeling of the tumor cells.

The cytotoxicity tests showed that 0.2 mg of anti-SL2 immunoglobulin caused lysis of 91% of the lymphoma cells in the presence of complement within 1 hour.

Rabbit-Anti-HPM

The rabbit-anti-HPM serum was absorbed with BALB/c tissue homogenates and used for the chlorambucil-antibody complex experiments. Further details about this serum have been described elsewhere (30).

Binding of Goat-Anti-SL2 Immunoglobulins to C57BL Peritoneal Exudate Cells

Hyperimmune C57BL mice were injected with 1 ml of immunoglobulins (0.2 mg in 1 ml of PBS) 1 hour before collection of the PECs. Fluorescence microscopy of the collected macrophages showed that these cells were labeled with immunoglobulins.

Physical Linkage of Chlorambucil to Immunoglobulin

The method of linking chlorambucil physically to the isolated immunoglobulin fractions of the sera has been described elsewhere (30).

After the linking procedure the amounts of (active) chlorambucil per ml and protein per ml were determined. The amount of protein per ml after the linking procedure with chlorambucil was determined according to the method of Lowry et al (31). Protein concentrations were 0.816 and 0.600 mg/ml in the complex with anti-HPM antibodies and anti-SL2 antibodies respectively. The protein concentrations were 0.660 and 0.550 mg/ml in the complexes with the corresponding control globulins.

The active and the hydrolyzed forms of chlorambucil in the complexes were measured by a method described by Linford et al (32). After complexing the drug with anti-HPM immunoglobulins, 0.237 mg of active chlorambucil per mg of protein was linked; 0.712 mg of active chlorambucil per mg of protein was linked in the complex with anti-SL2 immunoglobulins. After complexing with the control immunoglobulins (from normal rabbits), 0.298 and 0.412 mg of active chlorambucil per mg of protein was linked in the control of anti-HPM and anti-SL2 immunoglobulin respectively.

The dose of the drug and/or protein in the "control" solutions was adjusted to the determined values.

Chlorambucil

Chlorambucil was obtained from Sigma Chemical Co, St. Louis, Mo.

Bacillus Calmette-Guérin

Live BCG organisms were received weekly by plane from the Institut Pasteur, Paris, France. The organisms were stored at 4°C. The BCG was used within 48 hours after arrival.

DTIC

DTIC was obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, and was stored at −20°C. Two mg of DTIC/mouse/day was injected sc, ip, or iv (tail vein). The injected volume was 0.2 ml.

DTIC was dissolved in Fischer's medium. The pH was adjusted to 7.2. The tail was badly damaged after several weeks by the daily injections. The influence of iv injections of DTIC on the numbers of leukocytes and thrombocytes in the peripheral blood of BALB/c mice was measured. This gives an indication of the influence of the drug on the cellular immune system. The test mice (female BALB/c, 10–15 weeks old) were injected iv daily (5 days a week) for 6 weeks with 2 mg of DTIC, pH 7.2. Once a week the number of leukocytes and thrombocytes was counted in four mice. These mice were not injected on the same day that the blood was collected. The mice were discarded after collection of the blood. Control mice received injections with 0.2 ml of Fischer's medium. The blood was obtained by orbita puncture and collected by hematocrit tubes with EDTA. The results of these cell counts show that DTIC has no influence on the number of leukocytes and thrombocytes in the peripheral blood.

Cytotoxicity

Cytotoxicity was expressed as $CI = (N–T)/N \times 100$, where CI = cytotoxicity index; N = the number of target cells in the control; and T = the number of cells in the test.

Complement

Guinea pig and rabbit complements were purchased from the Rijks Instituut voor de Volksgezondheid, Bilthoven, The Netherlands.

In Vivo Experiments

The in vivo experiments were carried out with five or ten mice per observation. BALB/c mice (injected with HPM cells) were regarded as cured if they were macroscopically free of tumor 100 days after challenge. The DBA/2 mice (injected with SL2 cells) were regarded as cured if they survived a period of 40 days after challenge under the condition that their survival time was significantly different (5% level) from the mean survival time of their test group.

TABLE 1.—Effect of treatment with C57BL immune or hyperimmune PECs on survival time of DBA/2 mice injected with SL2 cells (*from* ref 33)

Treatment	No. of SL2 cells used for challenge: 2×10^5 Survival time of non-cured mice (days)	2×10^5 No. of cured mice*	2×10^7 Survival time of non-cured mice (days)	2×10^7 No. of cured mice*
1 injection with				
Immune† cells	30.6 ± 2.8	0	14.6 ± 3.1	0
Hyperimmune‡ cells	22.5 ± 1.5	3	25.5 ± 2.9	1
Medium	13.5 ± 0.4	0	10.3 ± 2.0	0
3 injections with§				
Immune cells	28.5 ± 0.5	3	10.2 ± 0.2	0
Hyperimmune cells	23.7 ± 1.6	3	25.0 ± 5.0	1
Medium	13.8 ± 2.2	0	9.5 ± 0.5	0
5 injections with				
Immune cells	28.0 ± 3.7	2	14.0 ± 1.0	2
Hyperimmune cells	33.0	4	12.8 ± 1.6	0
Medium	12.0 ± 0.5	0	9.0 ± 0.0	0
8 injections with				
Immune cells	23.6 ± 4.5	0	14.2 ± 1.2	0
Medium	13.6 ± 0.8	0	10.0 ± 0.0	0
3 injections (within 24 hrs) with¶				
Immune cells	22.2 ± 2.9	0	10.6 ± 0.0	0
Hyperimmune cells	27.0	4	15.4 ± 2.7	0
Medium	12.5 ± 0.5	0	9.8 ± 0.4	0

*5 mice were used for each observation.

†Immune cells were obtained 10 days after immunization with 10^7 SL2 cells.

‡Hyperimmune cells were obtained from C57BL mice (hyperimmunized with 4 ip injections of 10^7 SL2 cells given at 9-day intervals) 5 days after the last immunization.

§The first injection was given 2 hrs after the SL2 cells and the following injections at 24-hr intervals.

¶The injections were given 2, 13, and 24 hrs after the SL2 cells.

RESULTS

DBA/2-SL2 Lymphosarcoma System

Allogeneic Immune and Hyperimmune Peritoneal Exudate Cells

DBA/2 mice inoculated with various doses of SL2 cells were treated with allogeneic immune or hyperimmune PECs. The injections consisted of 2×10^6 hyperimmune macrophages plus contaminating lymphocytes. More details about the experiments and the results are shown in table 1. From these results, it is clear that DBA/2 mice inoculated with up to 2×10^7 SL2 cells can be cured. It was further shown that treatment with nonimmune C57BL PECs had no therapeutic effect; no increase in survival time was obtained (27,33). Results of preliminary experiments (not published) in which higher numbers of PECs are used suggest that successful eradication of $\geq 10^8$ SL2 cells in DBA/2 mice might be achieved.

The peritoneal exudate comprises macrophages and an approximately equal number of lymphocytes. The question arose as to whether the therapeutic effect is due to macrophages, to lymphocytes, or to both. Thus, macrophages and lymphocytes were separated (as described in the Materials and Methods section) and were tested in mice injected ip with 2×10^5 SL2 cells. The results (table 2) show that hyperimmune macrophages and hyperimmune lymphocytes have some therapeutic value on their own. However, comparison with the results in table 1 shows that the best results are obtained with a mixture of macrophages and lymphocytes.

TABLE 2.—Therapy with immune or hyperimmune macrophages or lymphocytes (*from* ref 33)

Treatment	1 injection		3 injections*	
	Survival time of non-cured mice	No. of cured mice†	Survival time of non-cured mice	No. of cured mice†
2×10^6 immune peritoneal macrophages	—	—	15 ± 1	0
2×10^6 hyperimmune peritoneal macrophages	16 ± 2	0	18 ± 2	0
2×10^6 immune peritoneal lymphocytes	—	—	20.8 ± 0.6	1
2×10^6 hyperimmune peritoneal lymphocytes	27 ± 5	0	34 ± 2.0	1
Medium only	12.7 ± 0.5	0	12.0 ± 0	0

*The first injection was given 2 hrs after SL2 cells (2×10^5) and the following injections at 24-hr intervals.

†5 mice were used for each observation.

TABLE 3.—Therapy with immune and hyperimmune spleen cells of DBA/2 mice inoculated with SL2 cells* (*from* ref 33)

Treatment	1 injection		3 injections†	
	Survival time of non-cured mice	No. of cured mice‡	Survival time of non-cured mice	No. of cured mice‡
Immune spleen cells§	—	—	15 ± 1	0
Hyperimmune spleen cells	21 ± 1	1	27 ± 3	0
Medium	13 ± 0	0	12 ± 0	0

*DBA/2 mice injected ip with 2×10^5 SL2 cells.

†The first injection was given 2 hrs after inoculation with SL2 cells; the other injections were given at 24-hr intervals.

‡5 mice were used for each observation.

§4×10^6 cells.

Allogeneic Hyperimmune Spleen Cells

C57BL mice were immunized or hyperimmunized with SL2 cells as described in the Materials and Methods section. DBA/2 mice injected ip with 2×10^5 SL2 cells were treated with one or three injections with 4×10^6 hyperimmune spleen cells. The inferior results obtained with spleen cells (table 3) compared with PECs can be due to (*a*) the route of immunization (ip) which is of advantage for stimulation of PECs above spleen and lymph node cells, and (*b*) the percentage of macrophages in the spleen which is less favorable than that in the PEC suspension. The lymph node cells, which contain almost no macrophages, were totally ineffective (33).

Peritoneal Exudate Cells Coated With Immunoglobulins

Allogeneic C57BL immune and hyperimmune PECs were coated with goat-anti-SL2 immunoglobulin as described in the Materials and Methods section. Three injections of 2×10^6 of these macrophages plus the contaminating lymphocytes given at 1-day intervals were used as a therapy. Table 4 shows that binding of the cytophilic antibody to hyperimmune PECs impedes the cytotoxic action of these cells. The decreased in vivo cytotoxicity of coated immune and hyperimmune macrophages may be due to (*a*) the fact that in vivo the immunoglobulin makes the immune or hyperimmune PECs more non-self within the DBA/2 mice which might

TABLE 4.—Treatment with PEC coated with immunoglobulin (*from* ref 33)

Treatment†	No. of SL2 cells used for challenge*					
	2×10^5		2×10^6		2×10^7	
	Survival time of non-cured mice	No. of cured mice‡	Survival time of non-cured mice	No. of cured mice‡	Survival time of non-cured mice	No. of cured mice‡
Immune PECs	28.5 ± 0.5	3	21.0	4	10.2 ± 0.2	0
Immune PECs coated with anti-SL2 IgG	28.2 ± 3.2	0	28.4 ± 2.9	0	19.5 ± 3.2	0
Immune PECs coated with control IgG	14.2 ± 0.8	0	14.0 ± 0.7	1	11.4 ± 1.9	0
Hyperimmune PECs	23.7 ± 1.6	3	21.0 ± 2.0	3	25 ± 5.0	1
Hyperimmune PECs coated with anti-SL2-IgG	36.3 ± 3.6	1	25.6 ± 9.6	0	11.8 ± 2.0	0
Hyperimmune PECs coated with control IgG	13.8 ± 1.0	0	13.6 ± 1.0	0	9.8 ± 0.8	0
Medium	12.2 ± 3.4	0	11.3 ± 3.3	0	9.9 ± 3.1	0

*ip injection of the SL2 cells.
†3 ip injections with 2×10^6 peritoneal macrophages (contaminated with lymphocytes) were given at 2, 24, and 48 hrs.
‡5 mice were used for each observation.

be sufficient to prevent optimal function of the transferred cells (*b*) blocking of reactive sites of the PECs by the goat immunoglobulins resulting in abrogation of the cytotoxic effect of the immune cells or (*c*) competition between goat immunoglobulins and isologous cytophilic antibodies which might be present on the surface of immune macrophages.

Chlorambucil-Anti-SL2 Antibody Complexes

DBA/2 mice were injected ip with 2×10^5 SL2 cells. Treatment with the listed solutions did not result in prolongation of the survival time. The animals were treated with (*a*) chlorambucil anti-SL2 IgG complex, (*b*) a mixture of chlorambucil plus anti-SL2 IgG, (*c*) chlorambucil plus anti-SL2 IgG 2 hours later, or (*d*) anti-SL2-IgG plus chlorambucil 2 hours later.

Bacillus Calmette-Guérin

DBA/2 mice were injected ip with 2×10^5 SL2 cells. The animals were treated with one or six injections of 5 mg of BCG ($2–4 \times 10^7$ organisms) suspended in 1 ml of Fischer's medium. The first injection was given 2 hours after the tumor cells. The other injections were given at weekly intervals. The injections were ip or sc. There was no prolongation of survival time of the test mice compared to the survival of the controls whether they were treated ip with one (16.2 ± 1 days) or six (16.4 ± 1 days) injections or sc with one (18 ± 0 days) or six (19.5 ± 1 days) injections. The survival time of the controls was 18.4 ± 1 days.

DTIC

DBA/2 mice were injected ip with 2×10^5 SL2 cells. The mice were treated with injections of 2 mg of DTIC dissolved in 0.2 ml of Fischer's medium (pH 7.2). The injections were given sc or ip daily for 6 weeks (5 days a week). Control mice were injected with Fischer's medium. There was no significant prolongation of the survival time of the test mice sc (19.2 ± 2 days) or ip (14.8 ± 1 days) treated with DTIC compared to the survival time of the controls (17.6 ± 2 days).

BALB/c-Harding-Passey Melanoma System

Allogeneic Immune and Hyperimmune Peritoneal Exudate Cells

BALB/c mice inoculated ip with 10^7 HPM cells were treated with one or three ip injections with allogeneic immune or hyperimmune PECs comprising 2×10^6 macrophages plus contaminating lymphocytes. The first injection was given 2 hours after the tumor cells. The other injections were given at 24-hour intervals. It was shown that 10^7 HPM cells could be eradicated successfully after treatment with three successive injections with 10 days' immune PECs. Treatment with one or three injections with hyperimmune PECs was also effective. However, treatment with one or three injections with normal (nonimmune) PECs did not result in significant prolongation of the survival time (table 5).

TABLE 5.—Treatment of HPM-bearing* BALB/c mice with allogeneic immune and hyperimmune PECs†

Treatment	1 injection		3 injections‡	
	Survival time of non-cured mice	No. of cured mice§	Survival time of non-cured mice	No. of cured mice§
PECs				
Immune	31.2 ± 3	0	—	5
Hyperimmune	31.6 ± 4	2	47.5 ± 9	1
Normal	28.4 ± 7	0	37 ± 14	0
Medium	26.7 ± 6	0	24.2 ± 3	0

*BALB/c mice were injected ip with 10^7 HPM cells.
†2×10^6 macrophages contaminated with lymphocytes.
‡The first injection was given 2 hrs after the tumor cells; the other injections were given at 24-hr intervals.
§5 mice were used for each observation.

TABLE 6.—Treatment of HPM-bearing* BALB/c mice with allogeneic immune and hyperimmune spleen cells†

Treatment	1 injection		3 injections‡	
	Survival time of non-cured mice	No. of cured mice§	Survival time of non-cured mice	No. of cured mice§
Spleen cells				
Immune	45.2 ± 26	1	25.8 ± 5	0
Hyperimmune	—	5	71	4
Normal	27.8 ± 4	0	30.2 ± 7	0
Medium	26.7 ± 6	0	24.2 ± 3	0

*BALB/c mice were injected ip with 10^7 HPM cells.
†4×10^6 spleen cells.
‡The first injection was given 2 hrs after the tumor cells; the other injections were given at 24-hr intervals.
§5 mice were used for each observation.

Allogeneic Immune and Hyperimmune Spleen Cells

BALB/c inoculated ip with 10^7 HPM cells were treated with one or three ip injections with 4×10^6 allogeneic immune or hyperimmune spleen cells. The first injection was given 2 hours after the tumor cells. The other injections were given at 24-hour intervals. Optimal results were obtained after treatment with one or three injections with hyperimmune spleen cells. Treatment with normal (non-immune) spleen cells did not result in significant prolongation of the survival time (table 6).

Chlorambucil-Anti-HPM-Antibody Complexes

The results of the in vivo experiments have been described in detail elsewhere (30). Briefly, it was shown that 50% of BALB/c mice injected ip with 10^7 HPM cells and treated with five ip injections with the chlorambucil (anti-HPM)-antibody complex were macroscopically free of tumor after 120 days. The statistical analysis of the data showed that the treatment with the immune complex is significantly (5% level) better than treatment with any other solution tested.

Bacillus Calmette-Guérin

BALB/c mice injected ip with 10^6 HPM cells were injected weekly for 6 weeks with 0.05 mg of BCG ($2–4 \times 10^5$ living organisms) ip, sc, and iv. The first injection was given 2 hours after 10^6 tumor cells. BCG treatment resulted in prolongation of the survival time. The best results were obtained if the tumor and BCG were both injected ip, with four of five mice surviving. When the tumor was injected ip and BCG was given sc or iv, two and one of five mice were cured respectively.

DTIC

BALB/c mice were injected ip with 10^6 HPM cells. The mice were injected daily with DTIC (except during the weekends) for 6 weeks unless the ani-

mals died earlier. The first injection of DTIC (pH 7.2) was given 2 hours after injection of the tumor cells. The drawbacks of injection in the tail vein were clear. For this reason, we also studied the effect of ip and sc injections. Table 7 shows that there was a significant prolongation of the survival time compared with the controls not treated with DTIC. Controls injected with DTIC (not challenged with HPM cells) survived for > 100 days.

DISCUSSION

Various therapies have been tested in two mouse tumor systems: (*a*) the DBA/2-SL2 lymphosarcoma system and (*b*) the BALB/c-HPM system. We regard these systems as rather different since (*a*) the SL2 cells are syngeneic to DBA/2 mice, the tumor grows fast (10^7 SL2 cells injected ip kill a mouse after about 10–12 days), and the tumor grows ip as an ascitic tumor, and (*b*) HPM is a transplantable murine melanoma which is not syngeneic to BALB/c mice, the tumor grows rather slowly (10^7 HPM cells injected ip kill a mouse after about 30 days), and the tumor grows ip as a solid tumor.

The therapies tested were: allogeneic hyperimmune PECs (25–27), allogeneic hyperimmune PECs coated with xenogeneic immunoglobulin (34), allogeneic hyperimmune spleen cells (26,27,33), chlorambucil-antibody complexes (13,16,34), BCG (5–7), and DTIC (35–37).

This comparison of the antitumor effect of various therapies has been made (*a*) since the relative potency of each form of therapy was unknown, and (*b*) to see whether very different mouse tumor systems are sensitive to the same therapy or to different therapies. Since we have tested the therapies in only two different mouse tumor systems, we are aware that they might give different results in any other mouse tumor system and in man.

TABLE 7.—Therapeutic value of injection with 2 mg of DTIC, pH 7.2,* in BALB/c mice injected with HPM† cells

Treatment	Localization of the treatment	Survival time (days)	No. of cures‡
–		27 ± 3	0
+	sc	45 ± 9	0
–		25 ± 1	0
+	ip	46 ± 18	1
–		25 ± 1	0
+	iv	52 ± 17	0

*The mice were injected daily (5 days a wk) for 6 wks.
†5×10^6 HPM cells injected ip.
‡5 mice were used for each observation.

The results of our study are summarized in table 8. These results suggest that it is very easy to find antitumor effects in the BALB/c-HPM system since in this system each of the tested therapies led to prolongation of survival time and cures of mice injected ip with 10^6–10^7 tumor cells. The highest tumor dose tested was 10^7 cells injected ip. Higher doses were not tested since it is difficult to count such a high dose of HPM cells accurately. This means we do not know exactly the highest dose of injected HPM cells which can be eradicated by the therapies shown in table 8 (with the score +++).

On the other hand, in the DBA/2-SL2 system the therapies with immune spleen cells or immunoglobulin-coated hyperimmune PECs showed only a slight antitumor effect, while chlorambucil-antibody complex, BCG, and DTIC did not have a measurable antitumor effect in mice injected with 2×10^5 tumor cells. Immune PECs and hyperimmune PECs could eradicate up to 2×10^7 SL2 cells; higher doses of SL2 cells could not be eradicated.

Consequently, one mouse tumor system may be rather sensitive to therapy while another may be rather insensitive. It seems relevant with regard to this conclusion that after transplantation of tumor cells, 3% of 1500 BALB/c reject transplanted HPM cells (38), whereas rejection never occurs after ip transplantation of ≥ ten SL2 cells into DBA/2 mice (39).

These differences in sensitivity to a given therapy and in spontaneous tumor rejection might be due to (*a*) the difference in growth rate of the HPM and the SL2 tumor, (*b*) the difference in the degree of invasion by host macrophages of the HPM tumor (≥ 50%; ref 40) and the SL2 tumor (5%; Van Loveren H, unpublished results), and (*c*) the difference

TABLE 8.—Comparison of various therapies

Treatment	Effect of treatment* DBA/2-SL2	BALB/c-HPM†
Immune PECs	++++	+++
Hyperimmune PECs	++++	+++
Immune spleen cells	–	+++
Hyperimmune spleen cells	+	+++
Immunoglobulin-coated		
Immune PECs	–	ND‡
Hyperimmune PECs	+	ND‡
Chlorambucil-antibody complex	–	+++
BCG	–	++
DTIC	–	++

*No. of cells that can be eradicated successfully: – = $< 2 \times 10^5$; + = 2×10^5; ++ = 10^6; +++ = 10^7; ++++ = 2×10^7.
†For reasons of accuracy, the maximum dose of HPM cells used for challenge was 10^7.
‡ND = not done.

in antigenicity (major histocompatibility complex and tumor antigen).

We conclude that (*a*) the therapy with hyperimmune PECs is the most effective tested therapy in the DBA/2-SL2 system and is one of the most effective tested therapies in the BALB/c-HPM system, and (*b*) the BALB/c-HPM system seems much more sensitive to therapy than the DBA/2-SL2 system.

In this paper only local antitumor effects (with the exception of BCG and DTIC) were studied. We are aware of the limitations of such studies on these local effects and for this reason our group continues with research on the antitumor effects of therapies on tumors at a distant site.

REFERENCES

1. CURRIE GA. Eighty years of immunotherapy: a review of immunological methods used for the treatment of human cancer. Br J Cancer 26:141–153, 1972.
2. PROCTOR JW, LEWIS MG, and MANSELL PW. Immunotherapy for cancer: an overview. Can J Surg 19:12–22, 1976.
3. SINKOVICS JG. Progress in clinical immunotherapy for tumors. Postgrad Med 59:110–116, 1976.
4. GUTTERMAN JH. Cancer and systemic active immunotherapy today—prospects for tomorrow. Cancer Immunol Immunother 2:1–9, 1977.
5. MATHÉ G. Approaches to the immunological treatment of cancer in man. Br Med J 4:7–10, 1969.
6. GERMAIN RN, WILLIAMS RM, and BENACERRAF B. Specific and nonspecific antitumor immunity. II. Macrophage-mediated nonspecific effector activity induced by BCG and similar agents. J Natl Cancer Inst 54:709–720, 1975.
7. HAWRYLKO E. Immunopotentiation with BCG: dimensions of a specific antitumor response. J Natl Cancer Inst 54:1189–1197, 1975.
8. LIKHITE VV, and HALPERN BN. The delayed rejection of tumors formed from the administration of tumor cells mixed with killed *Corynebacterium parvum*. Int J Cancer 12:699–704, 1973.
9. MILAS L, HUNTER N, BASIC I, ET AL. Nonspecific immunotherapy of murine solid tumors with *Corynebacterium granulosum*. J Natl Cancer Inst 54:895–902, 1975.
10. WOODRUFF MFA, and DUNBAR N. Effect of local injection of *Corynebacterium parvum* on the growth of a murine fibrosarcoma. Br J Cancer 32:34–41, 1975.
11. SCHULTZ RM, PAPAMATHEAKIS JD, and CHIRIGOS MA. Direct activation in vitro of mouse peritoneal macrophages by pyran copolymer (NSC 46025). Cell Immunol 29:403–409, 1977.
12. SCHULTZ RM, PAPAMATHEAKIS JD, LUETZELER J, ET AL. Macrophage involvement in the protective effect of pyran copolymer against the Madison lung carcinoma (M109). Cancer Res 37:358–364, 1977.
13. GHOSE T, CERINI M, CARTER M, ET AL. Immunoradioactive agent against cancer. Br Med J 1:90–93, 1967.
14. CALENDI E, CONSTANZI G, INDIVERI F, ET AL. Histoimmunologic specificity of an anti-lymphoid tissue sarcoma gammaglobulin bound to methotrexate. Bull Chimico Farm 108:25–28, 1969.
15. MOOLTEN FL, CAPPARELL NJ, ZAJDEL SH, ET AL. Antitumor effects of antibody-diphtheria toxin conjugates. II. Immunotherapy with conjugates directed against tumor antigens induced by simian virus 40. J Natl Cancer Inst 55:473–477, 1975.
16. GHOSE T, NORVELL ST, GUCLU A, ET AL. Immunotherapy of cancer with chlorambucil carrying antibody. Br Med J 3:495–499, 1972.
17. DAVIES DAL, BUCKHAM S, and MANSTONE AJ. Protection of mice against syngeneic lymphomata: II. Collaboration between drugs and antibodies. Br J Cancer 30:305–411, 1974.
18. GHOSE T, GUCLU A, and TAI J. Suppression of an AKR lymphoma by antibody and chlorambucil. J Natl Cancer Inst 55:1353–1357, 1975.
19. ECCLES SA, and ALEXANDER P. Sequestration of macrophages in growing tumors and its effect on the immunological capacity of the host. Br J Cancer 30:42–49, 1974.
20. EVANS R, and ALEXANDER P. Cooperation of immune lymphoid cells with macrophages in tumour immunity. Nature 228:620–622, 1970.
21. RUSSELL SW, DOE WF, and COCHRANE CG. Number of macrophages and distribution of mitotic activity in regressing and progressing Moloney sarcomas. J Immunol 116:164–166, 1976.
22. BRUNNER KT, MAUEL J, RUDOLF H, ET AL. Studies of allograft immunity in mice. I. Induction, development and in vitro assay of cellular immunity. Immunology 18:501–513, 1970.
23. HELLSTRÖM I, HELLSTRÖM NE, SJÖGREN HO, ET AL. Demonstration of cell-mediated immunity to human neoplasms of various histological types. Int J Cancer 7:1–16, 1971.
24. BARTLETT PF, FENDERSON BS, and EDIDIN M. Inhibition of tumor growth mediated by lymphocytes sensitized in vitro to a syngeneic teratocarcinoma 402AX. J Immunol 120:1211–1217, 1978.
25. DULLENS HFJ, and DEN OTTER W. Therapy with allogeneic immune peritoneal cells. Cancer Res 34:1726–1730, 1974.
26. DULLENS HFJ, KINGMA FJ, and DEN OTTER W. Immunotherapy with allogeneic hyperimmune peritoneal cells in a murine lymphoma system. Eur J Cancer 10:41–47, 1974.
27. DULLENS HFJ, WOUTERSEN RA, DE WEGER RA, ET AL. Eradication of tumour cells by successive injections of allogeneic immune and hyperimmune peritoneal cells in a murine lymphoma system. Eur J Cancer 10:701–706, 1974.
28. STEWART HL, SNELL KC, DUNHAM LJ, ET AL. Harding-Passey melanoma, mouse. *In* Transplantable and Transmissible Tumors of Animals. Washington, DC, Armed Forces Institute of Pathology, 1959, pp 51–56.
29. FISCHER GA. Studies on the culture of leukemia in vitro. Ann NY Acad Sci 76:673–680, 1958.
30. DULLENS HFJ, DE WEGER RA, VENNEGOOR C, ET AL. Antitumour effect of chlorambucil-antibody complexes in a murine melanoma system. Eur J Cancer, 1978. In press.
31. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurement with the folin phenol reagent. J Biochem 193:265–275, 1951.
32. LINFORD JH, LEGAL J, and ZACHARIAS E. The construction of an ultra filtration unit to measure the binding of certain nitrogen mustards to serum proteins. Can J Biochem 40:137–146, 1962.
33. DULLENS HFJ. Cellular immunotherapy of murine tumors. Doctoral thesis. Utrecht State University, The Netherlands, 1975.
34. DULLENS HFJ, DE WEGER RA, WOUTERSEN RA, ET AL. Therapy with antibody coated immune and hyperimmune peritoneal cells in a murine lymphoma system. J Natl Cancer Inst 54:77–82, 1975.
35. SKIBBA JL, BEAL DD, RAMIREZ G, ET AL. *N*-demethylation of the antineoplastic agent 4(5)-(3,3-dimethyl-1-triazeno)imidazole-5(4)-carboxamide by rats and man. Cancer Res 30:147–150, 1970.

36. SAVLOV ED, HALL TC, and OBERFIELD RA. Intra-arterial therapy of melanoma with dimethyl triazeno imidazole carboxamide (NSC-45388). Cancer 28:1161–1164, 1971.
37. NEWLANDS ES, OON CJ, ROBERTS JT, ET AL. Clinical trial of combination chemotherapy and specific active immunotherapy in disseminated melanoma. Br J Cancer 34:174–179, 1976.
38. SUGIURA K. The effects of various physical and chemical factors on transplantable mouse melanoma. *In* Biology of Melanomas. New York, New York Academy of Sciences, 1948, vol 4, pp 369–387.
39. DEN OTTER W, RUNHAAR BA, RUITENBEEK A, ET AL. Site-dependent differences in rejection of tumor cells with and without preimmunization. Eur J Immunol 4:444–446, 1974.
40. LEJEUNE FJ. Etude des macrophages dans le melanome Harding-Passey. Rev Inst Pasteur 4:349–359, 1971.

Phase II Study of ICRF-159 in Refractory Metastatic Breast Cancer [1,2]

Richard H. Creech, [3,4,*] Paul F. Engstrom, [3,4] David T. Harris, [3] Robert B. Catalano, [3,5] and Robert E. Bellet [3,6,7]

SUMMARY

ICRF-159, at a dose of 300 mg/m^2, was given orally every 8 hours for nine doses every 21 days to 40 patients with metastatic breast cancer refractory to hormonal therapy and cyclophosphamide, methotrexate, 5-fluorouracil, and adriamycin chemotherapy. Two patients with soft tissue disease had short-lived partial responses. The hematologic toxicity was severe. Three patients required rbc transfusions. Four patients became septic at the nadir of leukopenia; two of these patients died while leukopenic. Two patients had platelet counts < 25,000/mm^3. All patients who were nonevaluable or who had life-threatening or lethal toxicity were nonambulatory. Since the 19 nonambulatory patients had a median survival of only 1.25 months as compared to 7 months in ambulatory patients, it is recommended that future phase II trials in chemotherapy-refractory breast cancer be limited to ambulatory patients. Although ICRF-159 has minimal antineoplastic effects, it is not recommended for further investigations in metastatic breast cancer, even at more hematologically tolerable doses of 250 mg/m^2 every 8 hours for nine doses.

[Cancer Treat Rep 63:111–114, 1979]

ICRF-159, a nonpolar derivative of ethylenediaminetetraacetic acid, was first synthesized at the Imperial Cancer Research Fund facilities in London, England. In preclinical studies it has been found to be active against L1210 leukemia (1), to inhibit Lewis lung carcinoma pulmonary metastases (2), and to have antitumor synergism with irradiation in the S180 sarcoma system (3). It has also been found to be synergistic with adriamycin, daunorubicin, and *cis*-dichlorodiammineplatinum(II) in the L1210 system and with cyclophosphamide in the Lewis lung carcinoma system (1,4).

In a phase I trial of ICRF-159, Bellet et al found the maximum-tolerated oral dose to be 1 g/m^2/day in divided doses every 8 hours for 3 days every 21 days (5). The major toxicity was hematologic. In a phase II study, Ahmann et al (6) treated 14 metastatic breast cancer patients with ICRF-159 at a dose of 750 mg/m^2/day for 3 days in divided doses every 8 hours at 4-week intervals. Their patients had no responses and a median survival of 9 weeks. The primary toxic effect was leukopenia, which resulted in one drug-related death. The phase II trial of ICRF-159 reported in this paper was also conducted in patients with advanced metastatic breast cancer.

PATIENTS AND METHODS

Forty patients with metastatic breast carcinoma refractory to hormonal therapy and standard chemotherapy were entered in this trial of ICRF-159 (supplied by the Cancer Therapy Evaluation Pro-

[1]Received Sept 14, 1978; revised Nov 13, 1978; accepted Nov 28, 1978.

[2]Supported in part by Public Health Service grants CN-45055, CA-06927, and CA-06551 (National Cancer Institute), and RR-05539 (Division of Research Resources) from the National Institutes of Health, Department of Health, Education, and Welfare, and by an appropriation from the Commonwealth of Pennsylvania.

[3]Department of Medicine, American Oncologic Hospital, The Fox Chase Cancer Center, Philadelphia, Pa.

[4]Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia.

[5]Philadelphia College of Pharmacy and Science, Pa.

[6]Department of Medicine, Temple University School of Medicine, Philadelphia, Pa.

[7]We wish to thank Dr. Edward Lustbader and Pam Whitford for their assistance with the statistical analyses.

[*]*Reprint requests to:* Richard H. Creech, MD, American Oncologic Hospital, The Fox Chase Cancer Center, Philadelphia, Pa 19111.

gram, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md). All patients were required to be refractory to conventional therapy, to have measurable metastatic disease, and to have the following laboratory values: hemoglobin ≥ 9 g/100 ml; wbc count ≥ 4000/mm^3; platelet count ≥ 100,000/mm^3; total bilirubin ≤ 2 mg/100 ml; SGOT ≤ 80 IU; and BUN ≤ 20 mg/100 ml. The patient population studied had the following characteristics: median age, 54 years (range, 35–80 years); median disease-free interval, 11 months (range, 0–100 months); and median interval between development of metastases and initiation of ICRF-159, 20 months (range, 3–132 months). Twenty-one patients were ambulatory and 19 were nonambulatory at the start of therapy. Eighty-three percent of the patients had had previous hormonal therapy (18% response). Twenty-five percent had received low-dose cyclophosphamide, adriamycin, methotrexate, and 5-FU (80% response); 63% had received low-dose cyclophosphamide, methotrexate, and 5-FU (40% response); and 73% had received secondary adriamycin (21% response). The 40 patients studied had multiple sites of metastatic disease involvement (mean, 2.3 sites). Twenty-seven patients had skin and/or subcutaneous disease; 17 had bone or bone marrow involvement; 17 had pleural, lymphangitic, or nodular pulmonary disease; 14 had hepatic metastases; 14 had metastatic nodal disease; five had unresected breast carcinomas; four had brain metastases; and four had peritoneal involvement.

After giving informed written consent, each patient was given ICRF-159 at a dose of 300 mg/m^2 orally at 8-hour intervals for nine doses. This therapy was repeated at 21-day intervals until evidence of metastatic disease progression was noted. In patients with wbc counts ≤ 2500/mm^3 or platelet counts ≤ 90,000/mm^3 on the day of re-initiation of therapy, treatment was withheld until the wbc count returned to ≥ 4000/mm^3 and/or the platelet count returned to ≥ 100,000/mm^3. Treatment was then resumed at a dose of 250 mg/m^2 every 8 hours for nine doses. All patients were evaluated for disease response and hematologic, gastrointestinal, dermal, and neurologic toxic effects at the initiation of each course of therapy. Palpable dermal, subcutaneous, nodal, hepatic, and intra-abdominal masses were measured in two dimensions and pulmonary parenchymal disease was similarly measured on chest roentgenograms. Patients with abnormal liver scans or bone surveys had repeat studies at 3-month intervals. Serum SGOT, LDH, alkaline phosphatase, BUN, calcium, and glucose were performed at the initiation of therapy and at 6-week intervals.

Patients were considered evaluable for toxicity if they were available for followup at 7 days after initiation of therapy and were considered evaluable for response if they were available for followup at 21 days. The criteria used for response were those adopted by the Eastern Cooperative Oncology Group. A partial response (PR) occurred when a decrease in tumor surface area of ≥ 50% occurred and lasted for at least 4 weeks without an increase in size of any area of known malignant disease or appearance of new areas of disease. Stabilization or PR in any organ system associated with a complete response in another organ system was considered a PR. Association of stable disease with areas of PR was also considered a PR. Stabilization indicated no significant change in measurable or evaluable disease for at least 4 weeks with no deterioration in performance status. A progression indicated a significant (> 25%) increase in the size of lesions present at the start of therapy or the appearance of new metastases. Patients with otherwise stable disease associated with a decrease in performance status were considered progressors as were any patients with progression at any site despite evidence of disease response elsewhere. Toxicity caused by ICRF-159 was assessed according to Eastern Cooperative Oncology Group criteria.

RESULTS

Of the 40 patients treated with ICRF-159, 38 were evaluable for toxicity and 37 for response. Two patients not evaluable for either toxicity or response died at outside hospitals of progressive metastatic disease on Days 8 and 11 after initiation of therapy. The patient evaluable for toxicity only died of treatment-related sepsis on Day 9.

Response

Two patients (5%) had PRs. The first patient had a PR of subcutaneous metastases at Day 21 which lasted for 1 month. The second patient had a PR of an extensive chest wall recurrence beginning on Day 42 and lasting for 4 months. Fourteen patients (38%) had stabilization of disease and 21 patients (57%) had progressive disease. It is interesting to note that neither of the ICRF-159 responders had previously responded to hormonal therapy or cyclophosphamide, methotrexate, 5-FU, and adriamycin chemotherapy.

Survival

The mean survival of the partial responders was 11 months, the median survival of the stable pa-

tients was 9 months (range, 3–22 months), and the median survival of the progressors was 3 months (range, 0.75–38.5 months). There were no statistically significant differences among the survival curves of these three groups when life-table analyses were performed (7).

When comparing the survivals of the patients by performance status, the 21 ambulatory patients had a median survival of 7 months (range, 2–27+ months) while the nonambulatory patients had a median survival of 1.25 months (range, 0.25–5 months). Using a life-table analysis (7) there was a statistically superior survival for the ambulatory patients beginning at Week 3 of therapy, with $P < 0.01$ using a two-tailed test (fig 1). The ambulatory patients received a mean of 5.7 courses of ICRF-159 (range, one to 26 courses) whereas the nonambulatory patients received a mean of 1.6 courses (range, one to four courses).

Toxicity

The toxic effects in the 38 evaluable patients during ICRF-159 therapy can be seen in table 1. The major toxicity was hematologic. Fifty-eight percent of the patients developed wbc counts < 3000/mm^3 and 16% of the patients developed platelet counts < 90,000/mm^3. Three patients required rbc transfusions. Two of four patients who became septic at the nadir of leukopenia survived their sepsis. Of the four evaluable patients who died within 1 month of initiation of therapy, two died of progressive metastatic disease unrelated to chemotherapy-induced toxicity. One patient died on Day 21 of rapidly progressive disease associated with ICRF-159-induced leukopenia (wbcs, 1000/mm^3), thrombocytopenia (platelets, 17,000/mm^3), mild mucosal bleeding, and fever. The fourth patient died of presumed sepsis with a wbc count of 600/mm^3 on Day 8. ICRF-159 caused alopecia in 20% of patients (29% of the 38 patients not being evaluable for this toxicity because of previous adriamycin therapy or whole-brain irradiation). Five percent of the patients complained of lethargy. No patient had renal, pulmonary, hepatic, or cardiac toxicity. There were no episodes of hypocalcemia. Of the 29 patients receiving more than one course of therapy, the average final hematologically acceptable dose of ICRF-159 was 250 mg/m^2 orally every 8 hours for nine doses every 3 weeks.

TABLE 1.—Toxic effects during ICRF-159 therapy in 38 evaluable patients

Toxic effect	No. of patients (%)
Hematologic	
Hemoglobin (g/100 ml)	
7.5–9.5	3 (8)
< 7.5	3 (8)
Wbcs (× 10^3/mm^3)	
3–4.5	7 (18)
2–2.9	10 (26)
1–1.9	9 (24)
< 1	3 (8)
Platelets (× 10^3/mm^3)	
50–89	4 (11)
25–49	0
0–24	2 (5)
Gastrointestinal	
Nausea	7 (18)
Mild vomiting	6 (16)
Severe vomiting	1 (3)
Alopecia	
Not evaluable	11 (29)
Mild	4 (10)
Severe	4 (10)
Neurologic	
Lethargy	2 (5)

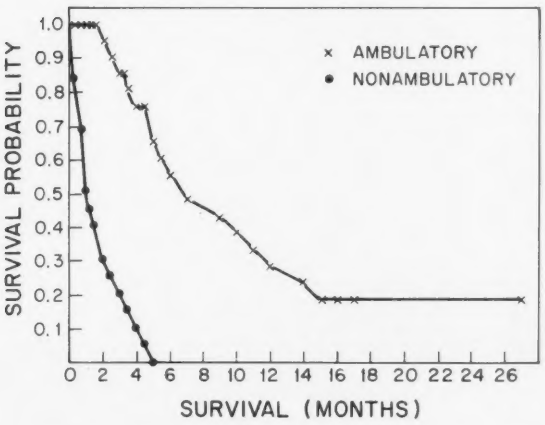


FIGURE 1.—Probability of survival related to ambulatory vs nonambulatory performance status: $P < 0.01$ beginning at 3 wks.

DISCUSSION

In this phase II study of ICRF-159 in 40 patients with metastatic breast cancer refractory to hormonal therapy and chemotherapy, 38 were evaluable for toxicity and 37 for antineoplastic response. Two patients with soft tissue disease had PRs. Neither had previously responded to conventional hormonal manipulation or chemotherapy. Their re-

sponses were short-lived and were not associated with improvement in survival.

The life-threatening risks (8% with wbcs < 1000/mm^3, 11% with sepsis, and 5% with platelets < 25,000/mm^3) and the 5% incidence of toxicity-associated death greatly outweighed the benefits (5% PRs). All of the patients in the study who were nonevaluable or who had life-threatening or lethal toxic effects were nonambulatory. Since there was a significant survival advantage for the ambulatory patients (median, 7 months) as compared to the nonambulatory patients (median, 1.25 months), it is recommended that future phase II studies in extensively previously treated metastatic breast cancer be limited to ambulatory patients.

Although ICRF-159 at a dose of 250 mg/m^2 orally every 8 hours for nine doses every 3 weeks is a hematologically more acceptable dosage regimen than a 300-mg/m^2 dose every 8 hours for nine doses in these previously chemotherapy-treated patients, the low response rate and short response durations do not justify further evaluation of this drug in metastatic breast cancer.

REFERENCES

1. WOODMAN RJ. Enhancement of antitumor effectiveness of ICRF-159 (NSC-129943) against early L1210 leukemia by combination with *cis*-diamminedichloroplatinum (NSC-119875) or daunomycin (NSC-82151). Cancer Chemother Rep Part 2, vol 4(1):45–52, 1974.
2. HELLMANN K, SALSBURY AJ, BURRAGE KS, ET AL. Drug-induced inhibition of hematogenously spread metastases. *In* Chemotherapy of Cancer Dissemination and Metastasis. New York, Raven Press, 1973, pp 355–359.
3. HELLMANN K, and MURKIN GE. Synergism of ICRF-159 and radiotherapy in treatment of experimental tumors. Cancer 34:1033–1039, 1974.
4. KLINE I. Potentially useful combinations of chemotherapy detected in mouse tumor systems. Cancer Chemother Rep Part 2, vol 4(1):33–43, 1974.
5. BELLET RE, MASTRANGELO MJ, DIXON LM, ET AL. Phase I study of ICRF-159 (NSC-129943) in human solid tumors. Cancer Chemother Rep 57:185–189, 1973.
6. AHMANN DL, O'CONNELL MJ, BISEL HF, ET AL. Phase II study of dianhydrogalactitol and ICRF-159 in patients with advanced breast cancer previously exposed to cytotoxic chemotherapy. Cancer Treat Rep 61:81–82, 1977.
7. THOMAS DR, and GRUNKEMEIER GL. Confidence interval estimation of survival probabilities for censored data. J Am Stat Assoc 70:865–871, 1975.

Ifosfamide Versus Cyclophosphamide in Combination Drug Therapy for Metastatic Breast Cancer [1,2]

Aman U. Buzdar,* Sewa S. Legha, Charles K. Tashima, Hwee-Y. Yap, Gabriel N. Hortobagyi, Evan M. Hersh, George R. Blumenschein, and Gerald P. Bodey [3,4]

SUMMARY

A combination of 5-fluorouracil (5-FU), adriamycin, and cyclophosphamide (FAC) was compared to a combination of 5-FU, adriamycin, and ifosfamide (FAI) in the treatment of metastatic breast cancer. All patients in the FAC and FAI groups also received nonspecific immunotherapy with bacillus Calmette-Guérin (BCG) and levamisole. Of 117 evaluable FAC patients, 19 (16%) achieved complete remission and 66 (56%) achieved partial remission. In the FAI group, eight of 49 (16%) evaluable patients achieved complete remission and 24 (50%) achieved partial remission. The response rates, durations of remission, and survival were similar in both groups. The combination of two immunotherapeutic agents (BCG and levamisole) did not have an additive effect since their results were similar to our previous experience with FAC-BCG and FAC-levamisole. In the FAI group, 25% of the patients had hematuria, while none of the patients in the FAC group had urinary complications. Nausea and vomiting were more severe in FAI-treated patients and some patients required iv fluids to correct dehydration. Ifosfamide in combination with 5-FU and adriamycin was more toxic than and not superior to cyclophosphamide.

[Cancer Treat Rep 63:115–120, 1979]

Adriamycin is the most effective single agent available for treating stage IV breast cancer (1). Various combinations of adriamycin, cyclophosphamide, vincristine, and 5-fluorouracil (5-FU) have produced response rates in the range of 70%–85% (2–5), of which 10%–20% have been complete remissions (CRs). At our institution we have been using a combination of 5-FU, adriamycin, and cyclophosphamide (FAC) which produces remission in 75% of patients with advanced breast cancer (3). The addition of nonspecific immunotherapy with bacillus Calmette-Guérin (BCG) or levamisole to this regimen has not improved the response rate but has modestly prolonged the durations of remission and survival (6,7). In this study a combination of 5-FU, adriamycin, ifosfamide (FAI), BCG, and levamisole was compared to FAC, BCG, and levamisole to determine whether or not the substitution of ifosfamide for cyclophosphamide reduces the myelosuppressive effect of the combination chemotherapy regimen, allows increased doses of 5-FU and adriamycin to be administered, and improves the treatment result.

Ifosfamide is a cyclophosphamide analog. Its chemical structure differs from cyclophosphamide in that the two functional chloroethyl groups are not attached to the same nitrogen. Studies of ifos-

[1]Received Sept 21, 1978; revised Nov 7, 1978; accepted Nov 16, 1978.

[2]Supported in part by contracts N01-CM-57042 (Division of Cancer Treatment) and N01-CB-33888 (Division of Cancer Biology and Diagnosis) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Departments of Medicine (Medical Breast Service) (*A. U. Buzdar, C. K. Tashima, H.-Y. Yap, G. N. Hortobagyi,* and *G. R. Blumenschein*) and Developmental Therapeutics (*S. S. Legha, E. M. Hersh,* and *G. P. Bodey*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4]We would like to thank Gay Canavan and Audrey Faye Grubbs for their help in the preparation of this manuscript.

**Reprint requests to*: Aman U. Buzdar, MD, Medical Breast Service, Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

famide in animal tumor systems have been especially encouraging, because it was more active than cyclophosphamide (8–10). Early clinical trials revealed that a much larger dose of ifosfamide could be given and that the major dose-limiting toxic effect of ifosfamide was hemorrhagic cystitis rather than myelosuppression, which is the dose-limiting toxic effect of cyclophosphamide (11,12). The bladder toxicity of ifosfamide is substantially reduced when the drug is administered by an iv infusion 1–2 hours daily for 5 days (13). Because ifosfamide was thought to be less myelosuppressive than cyclophosphamide, it was substituted for the latter drug in the FAC regimen for breast cancer. It was anticipated that this substitution would permit the administration of higher doses of each drug. The results reported here indicate that the substitution of ifosfamide for cyclophosphamide in the FAC regimen resulted in more toxicity and did not improve the therapeutic efficacy of the regimen.

MATERIALS AND METHODS

Between January 1976 and February 1977, all patients with histologically proven incurable carcinoma of the breast were considered eligible for this study. Patients were informed about the investigational nature of the study and were asked to sign a written informed consent according to the institutional guidelines. The patients were completely staged prior to initiation of treatment, and the workup included a complete history and physical examination, cbc, platelet and differential counts, urinalysis, and SMA-12 analysis which included alkaline phosphatase, SGOT, LDH, serum calcium, phosphorus, glucose, BUN, creatine, uric acid, and total bilirubin. All patients had adequate bone marrow function, as defined by a peripheral absolute granulocyte count of $> 2000/mm^3$ and a platelet count of $> 100,000/mm^3$. The staging evaluation also included chest roentgenogram, skeletal survey, liver scan, bone scan, and photographs of any skin lesions. All patients had an electrocardiogram prior to initiation of adriamycin therapy, which was repeated prior to each dose of adriamycin. Cbc and platelet and differential counts were done weekly to monitor the myelosuppressive toxicity of treatment. The SMA-12 and urinalysis were done prior to each cycle of chemotherapy. The patients receiving ifosfamide had daily urinalysis during the 5 days of treatment. The appropriate roentgenograms and scans were repeated every 3 months or earlier to evaluate response. Accurate measurements of soft tissue disease were made every 8 weeks.

Treatment Plan

All patients were to receive either ifosfamide or cyclophosphamide in combination with 5-FU and adriamycin. Patients also received nonspecific immunotherapy with BCG and levamisole. The treatment plan consisted of 5-FU at a dose of 500 mg/m^2 iv on Days 1 and 8, adriamycin at a dose of 50 mg/m^2 iv on Day 1 only, and cyclophosphamide at a dose of 500 mg/m^2 iv on Day 1. Patients who were randomized to the ifosfamide arm received ifosfamide at a dose of 800 mg/m^2 iv daily for 5 days. Ifosfamide was given in 500 ml of 5% dextrose in water over a 2–3-hour period. BCG (6×10^8 organisms) was given by scarification to all patients on Days 9, 13, and 17 of each 21-day cycle, along with levamisole at a dose of 100 mg/m^2 orally on Days 9, 10, 13, 14, 17, and 18 of each cycle. The courses of chemoimmunotherapy were repeated every 21 days. Adriamycin was discontinued at a dose of 450 mg/m^2 and patients were switched to a maintenance treatment regimen consisting of 500 mg/m^2 of 5-FU orally on Days 1 and 8, 30 mg/m^2 of methotrexate im on Days 1 and 8, and 500 mg/m^2 of cyclophosphamide orally on Day 2 of each 21-day cycle. BCG and levamisole were continued in the same dose and schedule as outlined above. Patients in the FAC and FAI groups received the same maintenance regimen as outlined above.

A total of 132 patients were entered in the FAC arm and 60 were entered in the FAI arm. This study was designed as a prospective randomized comparative trial: FAC versus FAI. However, the design of the study could not be adhered to because of frequent supply problems with ifosfamide. In the later part of the study, the FAI arm was closed because of increased bladder toxicity observed with ifosfamide in spite of the 5-day infusion schedule. This resulted in a greater number of patients in the FAC arm.

Dose Modification and Response Criteria

Doses of all three drugs were reduced by 20% if a patient's absolute granulocyte count dropped to $< 1000/mm^3$ or the platelet count dropped to $< 75,000/mm^3$. Doses of all three drugs were also decreased by 25% irrespective of blood cell count if the patients developed infectious complications. The doses of all chemotherapeutic agents were escalated by 20% if the patient's lowest absolute granulocyte count was $> 2000/mm^3$ and the platelet count was $> 100,000/mm^3$ during the previous course of chemotherapy. The dose of adriamycin was reduced by 50% if the patient's bilirubin was > 1 but < 3 mg/dl and by 75% if the bilirubin was > 3 mg/dl. Adria-

mycin was discontinued if there was a ≥ 30% decrease in the summed QRS voltage in the six standard limb leads on the electrocardiogram or if a patient showed signs or symptoms of congestive heart failure. If a patient developed hemorrhagic cystitis due to cyclophosphamide or ifosfamide, the drug was replaced by melphalan at a dose of 15 mg/m² orally on Day 1. A minimum of two cycles were required for a patient to be considered to have received an adequate trial for evaluating the efficacy of the treatment, but all patients were evaluated for toxicity.

CR was defined as a disappearance of all clinical evidence of the disease, including recalcification of lytic bone lesions and relief of tumor-related symptoms. Partial remission (PR) was defined as a reduction of > 50% in all measurable tumors without an increase in the size of any lesions or the appearance of any new lesions. Stable disease was defined as a tumor reduction of < 50% in measurable tumor size for at least 2 months without the appearance of any new lesions. Durations of response and survival were measured from the onset of treatment. Statistical methods used included a generalized Wilcoxon test (14) with a two-tailed analysis for testing differences between remission and survival curves and the Kaplan and Meier method for calculating and plotting remission and survival curves (15).

RESULTS

A total of 192 patients were entered in this study. Of these patients, 117 were evaluable in the FAC arm of the study, and 15 were inevaluable for the following reasons: four died before completing the first cycle of treatment, seven were lost to followup, and four had an inadequate trial, refusing further therapy after one cycle of chemotherapy. Sixty patients were entered in the FAI arm of the study. Eleven patients were considered inevaluable for the following reasons. Two patients died within the first 3 weeks of the study and two were lost to followup. Ifosfamide was discontinued after the first cycle of chemotherapy in five patients because of gross hematuria. One patient was ineligible because she had received extensive prior chemotherapy with DTIC and melphalan for melanoma, and one patient developed seizures during the first course of chemotherapy due to severe vomiting and hyponatremia possibly related to ifosfamide; the drug was subsequently discontinued.

As shown in table 1, the pretreatment characteristics of both groups were comparable. Of the 117 evaluable patients in the FAC arm, 85 (72%) achieved a CR or PR. Of 49 patients in the FAI arm, 32 (66%) achieved a CR or PR. The difference in the remission rates between the two groups was not statistically significant. The responses according to site of disease were also comparable (table 2), as was the median time to achieve remission from

TABLE 1.—Patient characteristics

	Therapy	
	FAC	FAI
No. of evaluable patients	117	49
Age in yrs		
Median (range)	51 (26–71)	52 (31–76)
% of patients		
Race		
White	87	78
Black	3	12
Latin American	10	10
Menopausal status		
Premenopausal	14	8
Postoophorectomy	25	29
Postmenopausal	61	63
Prior therapy		
Hormonal	48	45
Chemotherapy	10	8
Dominant disease site		
Soft tissue	6	8
Osseous	32	27
Visceral	62	63
No. of disease sites		
1–2	71	75
≥ 3	29	25
Disease-free interval in mos		
Median (range)	18 (0–276)	17 (0–108)

TABLE 2.—Overall response rates and response by site of metastases

	Therapy	
	FAC	FAI
No. of evaluable patients	117	49
No. of responses (%)		
CR	19 (16)	8 (16)
PR	66 (56)	24 (50)
Stable disease	27 (23)	14 (29)
Progressive disease	5 (4)	3 (6)
Overall remission	85 (72)	32 (66)
Median duration of responses (mos)		
CR	18	20
PR	14	16
Stable disease	8	6
Response by site of metastases (%)		
Soft tissue	80	68
Bone	58	67
Lung	70	75
Pleura	69	54
Liver	51	57

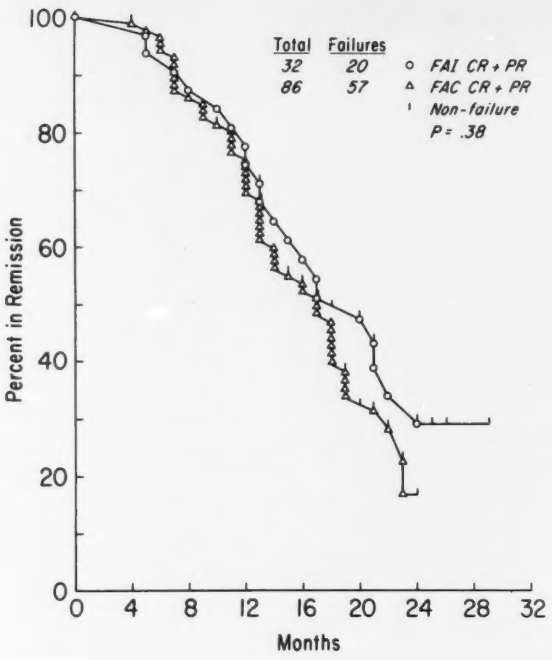


FIGURE 1.—Duration of remission with FAI-BCG and levamisole vs FAC-BCG and levamisole.

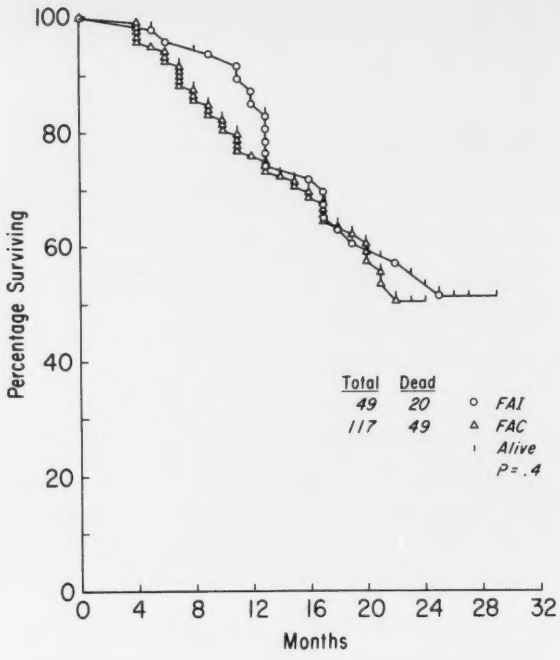


FIGURE 2.—Survival of evaluable patients in FAI-BCG and levamisole vs FAC-BCG and levamisole.

initiation of chemotherapy (median, 12 weeks; range, 4–50 weeks). The median duration of remission was 17 months for the FAC group and 17.8 months for the FAI group (fig 1). Patients achieving CR with either regimen had a slightly longer duration of remission than patients achieving PR, but the differences were not significant ($P = 0.1$ and 0.5, respectively). However, patients who achieved a PR had significantly longer durations of response than patients with stable disease ($P = 0.02$ and 0.02, respectively).

Figure 2 shows the overall survival of patients in both groups. The estimated median survival was 21.4 months for the FAC group and 23.5 months for the FAI group ($P = 0.4$). At 12.9 months in the FAC group and at 13 months in the FAI group, 25% of the patients had died of progressive disease.

Toxicity

The non-hematologic toxicity of both groups is shown in table 3. In spite of adequate hydration, 15 patients receiving ifosfamide developed hematuria. Ifosfamide had to be discontinued in five of these patients. The most common side effects included nausea and vomiting which were observed in 98% of the patients in both groups. However, four patients who received ifosfamide had severe nausea and vomiting leading to mild dehydration and requiring iv fluids. In one patient, persistent nausea

TABLE 3.—Comparative non-hematologic toxicity of FAC and FAI

	No. of patients (%) receiving—	
	FAC	FAI
Total patients evaluable for toxicity	132	60
Toxic effect		
Hematuria		
Gross	0	7 (12)
Microscopic	0	8 (13)
Congestive heart failure	3 (2)	4 (7)
Sepsis	10 (8)	2 (3)
Severe hyponatremia with seizures	0	1 (2)
Dehydration due to severe vomiting	0	3 (5)

TABLE 4.—Comparative hematologic toxicity of FAC and FAI

	FAC cycle—		FAI cycle—	
	1	6	1	6
Lowest absolute granulocyte count (× 10^3/mm^3) Median (range)	0.8 (0–3.2)	1.1 (0.3–4.1)	0.3 (0–2.5)	0.9 (0–2.0)
Lowest platelet count (× 10^3/mm^3) Median (range)	201 (32–450)	187 (45–497)	205 (28–817)	156 (42–480)
% of calculated drug dose administered Median (range)	100 (70–100)	80 (50–100)	100 (80–100)	80 (60–100)

and vomiting led to severe hyponatremia and seizures. The degree of myelosuppressive toxicity with the two regimens is shown in table 4. The degrees of myelosuppression with cycles 1 and 6 were similar, except that patients receiving FAI had somewhat lower granulocyte counts during the first cycle. Except for three patients in the FAI group, none of the other patients' drug doses could be escalated as planned in the study. Levamisole was discontinued in eight patients because of skin rash or neurologic symptoms (headache, dizziness, depersonalization or manic depressive state). BCG was discontinued in three patients: in two patients because of extensive local reactions in spite of dose reduction and in one patient who refused to continue BCG for cosmetic reasons.

DISCUSSION

The object of this study was to determine whether substitution of ifosfamide for cyclophosphamide in the FAC regimen would decrease the myelosuppressive toxicity, thus allowing dose escalations and possibly improving the response rate. The results show that the response rate and remission duration were similar in the two groups. The planned dose escalations could not be accomplished since ifosfamide did not reduce the myelosuppressive toxicity of the regimen. The observed response in the FAC and FAI groups was comparable to that reported with other combinations containing adriamycin and cyclophosphamide (2–5). These results indicate that the therapeutic efficacy of ifosfamide is similar to that of cyclophosphamide and that this drug had no advantage over cyclophosphamide in this dose schedule in the treatment of metastatic breast cancer.

The overall toxicity with the FAI regimen was more marked. Hematuria was observed in about 25% of the patients treated with this drug, while none of those treated with cyclophosphamide had urinary complications. With the 5-day schedule of ifosfamide, as used in this study, the bladder toxicity was significantly reduced, compared to the reported experience with the single dose, in which hemorrhagic cystitis has been observed in approximately 60% of the patients (11,12). Gastrointestinal toxic effects in the form of nausea and vomiting were more severe in ifosfamide-treated patients, with some requiring supplemental iv fluids to correct dehydration.

This study was designed as a prospective randomized study, but because of frequent supply problems with ifosfamide, a larger proportion of patients were treated with the FAC regimen. Similar problems could be anticipated in other studies in which an adequate supply of drugs is not on hand at the initiation of a clinical trial.

The results of this study compared to our previous experience with FAC in combination with BCG (6) or levamisole (7) did not show any difference in remission rate or duration of response. This suggests that the combination of two immunotherapeutic agents (BCG and levamisole) did not produce an additive effect. Since the durations of remission and survival are superior to those previously reported with FAC without immunotherapy (3), these data, again, support the idea that nonspecific immunotherapy is beneficial in the treatment of metastatic breast cancer. In conclusion, this study shows that ifosfamide in combination with 5-FU and adriamycin in the treatment of metastatic breast cancer is not less toxic and is not superior to cyclophosphamide.

REFERENCES

1. GOTTLIEB JA, RAVKIN SE, SPIGEL SC, ET AL. Superiority of adriamycin over oral nitrosoureas in patients with advanced breast carcinoma. Cancer 33:519–526, 1974.
2. BULL JM, TORMEY DC, LI S-H, ET AL. A randomized comparative trial of adriamycin vs. methotrexate in combination drug therapy. Cancer 41:1649–1657, 1978.

3. BLUMENSCHEIN GR, CARDENAS JO, FREIREICH EJ, ET AL. FAC chemotherapy for breast cancer. Proc Am Assoc Cancer Res and ASCO 15:193, 1974.
4. JONES SE, DURIE BG, and SALMON SE. Combination chemotherapy with adriamycin and cyclophosphamide for advanced breast cancer. Cancer 36:90–97, 1975.
5. SMALLEY RV, CARPENTER J, BARTOLUCCI A, ET AL. A comparison of cyclophosphamide, adriamycin, 5-fluorouracil (CAF) and cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, prednisone (CMFVP) in patients with metastatic breast cancer: a Southeastern Cancer Study Group project. Cancer 40:625–632, 1977.
6. GUTTERMAN JU, CARDENAS JO, BLUMENSCHEIN GR, ET AL. Chemoimmunotherapy of disseminated breast cancer: prolongation of remission and survival. Br Med J 2:1222–1225, 1976.
7. HORTOBAGYI GN, YAP HY, BLUMENSCHEIN GR, ET AL. Response of disseminated breast cancer to combined modality treatment with chemotherapy and levamisole with or without bacillus Calmette-Guérin. Cancer Treat Rep 62:1685–1692, 1978.
8. BROCK N. Pharmacological studies with ifosfamide—a new OXa2 phosphorine compound. *In* Proceedings of the 7th International Congress of Chemotherapy, Prague, 1971 (Hejzlar M, Semonsky M, and Meek M, eds). Munich, Urban and Schwarzenberg, 1972, pp 748–756.
9. VALERIOTE FA, BRUCE WR, and MEEKER BE. Synergistic action of cyclophosphamide and 1,3-bis(2-chloroethyl)-1-nitrosourea on transplanted murine lymphoma. J Natl Cancer Inst 40:935–944, 1968.
10. Summary of Information for Clinical Investigators. Brackwede, Germany, Asta-Werke AG, Chemische Fabrik, 1969.
11. SCHEEF W. Problems, experience, and results of clinical investigators with ifosfamide. *In* Proceedings of the 7th International Congress of Chemotherapy, Prague, 1971 (Hejzlar M, Semonsky M, and Meek M, eds). Munich, Urban and Schwarzenberg, 1972, pp 797–800.
12. BREMNER DN, MCCORMICK JSTC, and THOMSON JWW. Clinical trial of isophosphamide (NSC-109724)—results and side effects. Cancer Chemother Rep 58:889–893, 1974.
13. RODRIGUEZ V, BODEY GP, FREIREICH EJ, ET AL. Reduction of ifosfamide toxicity using dose fractionation. Cancer Res 36:2945–2948, 1976.
14. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–223, 1965.
15. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457–481, 1958.

Adriamycin: Comparison of a 5-Week Schedule With a 3-Week Schedule in the Treatment of Breast Cancer [1,2]

Brief Reports and Preliminary Communications

Edward W. Knight,[3,*] John Horton,[4] Thomas Cunningham,[4] Francis Rhie,[5] Stephen Lagakos,[6] Charles Rosenbaum,[4] Samuel Taylor IV,[4] and Juanita Tennant[3]

Adriamycin is the most useful broad-spectrum anticancer agent that has been developed in recent years (1). Its major limiting factor is cumulative dose-related cardiomyopathy. To date, no predictable method of ameliorating or preventing this life-threatening complication has been firmly established (1). After nearly 10 years of study, the safest approach still remains the limiting of total dose to 450–550 mg/m^2 of body surface area (2). The generally accepted best dosage regimen of adriamycin has been and is 60–70 mg/m^2 every 3 weeks. Although other regimens have been explored (3–5), none has been found to have unquestionable superiority in terms of toxicity, remission induction, maintenance of remission, or survival.

The purpose of this dose-schedule comparison was to determine if a 5-week schedule of adriamycin might be equivalent to the standard 3-week regimen in the treatment of far-advanced breast cancer. If shown to be of equal value, almost twice as much treatment time could be offered to responding patients before cardiotoxicity would become a significant risk.

PATIENTS AND METHODS

The criteria for entry in the study included the presence of progressive, inoperable breast malignancy previously untreated with adriamycin. Additional criteria for patient selection included a wbc count $> 5000/mm^3$, a platelet count $> 125,000/mm^3$, an expected survival of ≥ 2 months, a performance status of < 4, and adequate renal and hepatic functions. Patients with angina pectoris, frequent premature ventricular contractions, or other pronounced cardiac abnormalities were excluded.

Thirty-six evaluable patients with advanced breast cancer were entered in the study. Sixteen were randomized by the closed-envelope technique to a 3-week schedule and 20 were randomized to a 5-week schedule. Five patients on the every-3-week schedule and eight patients on the every-5-week schedule were initially ambulatory. Eleven patients on the every-3-week schedule were initially nonambulatory opposed to 12 patients on the every-5-week schedule. All but two patients had received previous chemotherapy, and all but six had also received previous radiation therapy. All 36 patients received a dose of 70 mg/m^2 of adriamycin. The maximum dose received by any patient was 550 mg/m^2.

TOXICITY

The toxicity experienced in this study was comparable to that reported in all previous publications (1–7). Partial or total alopecia occurred in almost 100% of the patients. Mild nausea and vomiting occurred in most patients after treatment. Dosage modification was not required because of gastrointestinal toxicity. On the every-3-week schedule, 25% of the patients demonstrated mild-moderate leukopenia and 10% experienced severe leukopenia. On the every-5-week schedule, 16% of the patients demonstrated mild-moderate leukopenia and 5% experienced severe leukopenia.

Thrombocytopenia was infrequent with both schedules. Wbc and platelet counts were required prior to each treatment and 100% of the calculated dose was administered if the wbc count was $> 5000/mm^3$ and the platelet count was $> 125,000/mm^3$. Fifty percent of the calculated dose was administered if the wbc count was 3500–4999/mm^3 or if the platelet count was 100,000–124,999/mm^3. Treatment was withheld if the wbc count was $< 3500/mm^3$ or if the platelet count was $< 100,000/mm^3$. The drug was

[1]Received Mar 21, 1978; revised Oct 12, 1978; accepted Oct 31, 1978.

[2]Supported by a cancer research grant from the Berkshire Medical Center, by The Nancy Newton McKelvey Memorial Fund, and by the Baptist Hospital Foundation.

[3]Department of Medical Oncology, Baptist Hospital, Pensacola, Fla.

[4]Albany Medical College, NY.

[5]Berkshire Medical Center, Pittsfield, Mass.

[6]Sidney Farber Cancer Institute and the Department of Biostatistics, Harvard University, Boston, Mass.

[*]*Reprint requests to:* Edward W. Knight, MD, Division of Oncology, Baptist Hospital, 1000 W Moreno St, Pensacola, Fla 32501.

not administered until the above values were achieved. Both schedules required dose reductions because of leukopenia following one or more courses of therapy. Treatment was also omitted if stomatitis or electrocardiogram abnormalities occurred and was not administered until the pretreatment status returned. There were no drug-related deaths and no patient developed cardiotoxicity. Cessation of treatment because of toxicity was not necessary. The toxic effects encountered on the two schedules were similar except for leukopenia which was slightly more frequent on the 3-week schedule. One patient on the every-5-week schedule received the maximum dose of 550 mg/m^2. All other responders exhibited progression of disease before this dose was reached.

TUMOR RESPONSE

The overall response rates for the every-3-week and the every-5-week schedules were 44% (seven of 16 patients) and 45% (nine of 20 patients) respectively. This includes two patients in each group who experienced complete responses, with the remaining experiencing partial responses. The percentages for those patients demonstrating stable disease on the every-3-week and the every-5-week schedules were 25% and 35% respectively. The overall progression rates for the every-3-week and the every-5-week schedules were 31% and 20% respectively. Partial response was defined as a $\geq 50\%$ decrease in the cross-sectional area of measurable lesions.

Progression was defined as the occurrence of any new lesion or an increase of any measurable lesion by $> 50\%$ over the smallest tumor size present during the study. The median time from the start of treatment until the start of response was 26 days (every-3-week schedule) and 55 days (every-5-week schedule). The number of courses until response occurred was nearly identical for the two schedules. The median response durations were 125 days (every-3-week schedule) and 159 days (every-5-week schedule).

SURVIVAL

The survival experiences of the two treatment groups were not significantly different, with median survivals of 26 weeks (every-3-week schedule) and 30 weeks (every-5-week schedule). As expected, responders survived longer than nonresponders, with corresponding medians of 36 and 20 weeks. On the other hand, it was rather surprising that initially ambulatory patients did not survive significantly longer than initially nonambulatory patients. Neither age, prior therapy, nor time from first diagnosis until start of treatment was correlated with survival time.

DISCUSSION

Adriamycin is an extremely valuable drug in the treatment of far-advanced breast cancer (6,7). This study suggests that a 5-week regimen may be equivalent to a 3-week regimen in terms of remission induction, maintenance of remission, toxicity, and survival. The longer interval between treatments on the 5-week schedule offers more convenience and less expense per interval of time, and may allow for almost twice as much treatment time for responding patients before cardiotoxicity becomes a significant risk. This schedule also offers more safety since there would be less frequent exposure to possible systemic and local toxicity. It is suggested that further studies comparing these and longer dosage regimens of adriamycin should be undertaken with larger patient populations.

REFERENCES

1. CROOKE ST. Antitumor Antibiotics, Medical Oncology Review Course Outline Book. Philadelphia, American College of Physicians, 1977, pp 472–510.
2. MINOW RA, BENJAMIN RS, LEE ET, ET AL. Adriamycin cardiomyopathy—risk factors. Cancer 39:1397–1402, 1977.
3. O'BRYAN RM, BAKER LH, GOTTLIEB JA, ET AL. Dose response evaluation of adriamycin in human neoplasia. Cancer 39:1940–1948, 1977.
4. WEISS AJ, METTER GE, FLETCHER WS, ET AL. Studies on adriamycin using a weekly regimen demonstrating its clinical effectiveness and lack of cardiac toxicity. Cancer Treat Rep 60:813–822, 1976.
5. KNIGHT EW, HORTON J, REILLY C, ET AL. A comparison of weekly vs 3 week course adriamycin in solid tumors. Proc Am Assoc Cancer Res 13:92, 1972.
6. TORMEY DC. Adriamycin (NSC-123127) in breast cancer: an overview of studies. Cancer Chemother Rep Part 3, vol 6(2):319–327, 1975.
7. HOOGSTRATEN B. Adriamycin (NSC-123127) in the treatment of advanced breast cancer: studies by the Southwest Oncology Group. Cancer Chemother Rep Part 3, vol 6(2):329–334, 1975.

New Side Effect of the Hypoxic Cell Sensitizer, Misonidazole [1,2]

Brief Reports and Preliminary Communications

Janet Partington, [3] Don Koziol, [3] Don Chapman, [3] Harvey Rabin, [4] and Raul C. Urtasun [3,*]

SUMMARY

As part of a phase II study with the hypoxic cell radiosensitizer, misonidazole, we have encountered two cases of dermatitis. When all drugs were withdrawn and the patients were challenged with a small single dose of misonidazole, identical adverse reactions reappeared demonstrating a drug-related skin hypersensitivity.

[Cancer Treat Rep 63:123–125, 1979]

A clinical phase II study of oral misonidazole (synthesized by Roche Products, Ltd) is in progress at our center. The aims of the study are to investigate the pharmacokinetics, toxicity, and radiosensitizing properties of this drug.

To date, previous and ongoing studies have documented mild to severe peripheral neuropathies occurring as toxic effects (1–4). Drug-related skin reactions have only recently been described. [5] This is a report of two patients who developed a disseminated maculopapular rash during treatment with misonidazole.

Patient 1

Patient 1 (a 68-year-old Caucasian female) was diagnosed as having a grade III astrocytoma in the left posterior parietal region. On January 16, 1978, a treatment plan of radiotherapy was initiated: 5800 rads in 30 daily fractions over 6 weeks. She was given 2 g of misonidazole three times a week for the first 3 weeks and 4 ½ hours prior to each radiation fraction.

Concomitant medications were dexamethasone, diphenylhydantoin, and phenobarbital. Prochlorperazine, 10 mg iv, was given ½ hour prior to each dose of misonidazole.

The first five doses, a total of 10 g in 2 weeks, were well-tolerated. Approximately 3 hours after receiving the sixth dose of misonidazole, an erythematous maculopapular rash appeared on the abdomen, spreading to the upper torso and the lower limbs. The patient complained of severe pruritus. All medications were discontinued. Four hours later, the patient was febrile with a temperature of 38°C. Diphenhydramine HCl was prescribed for the pruritus, although it was discontinued later because of drowsiness. Acetaminophen was administered for the patient's high temperature. Twenty-four hours later, the dermatitis had extended over her entire body and her temperature rose to 40.5°C. Dexamethasone was increased. On the premise that a gram-negative infection had developed, cephalexin and gentamicin were started. These drugs were discontinued 2 days later when all of the blood, urine, and sputum culture results were negative.

Within 48 hours of drug ingestion, the patient became afebrile. However, the rash and pruritus remained unchanged. Calamine lotion and Aveno baths were the treatment for the succeeding 12 days. After 7 days, the rash began to fade. Three days later, exfoliation occurred. With the resolution of the rash, petechiae appeared on the feet and calves which cleared within a few days. Radiation therapy was still continuing.

After 3 weeks, the patient's condition was stable and there was no dermatitis present. With the patient's consent, a challenge dose of 500 mg of misonidazole was administered. The only concomitant medication was dexamethasone. Ten hours after drug ingestion, a similar erythematous maculopapular rash developed over the patient's entire body excluding the palms and soles. Her tempera-

[1]Received July 21, 1978; accepted Nov 27, 1978.

[2]Supported by grant 55-45033 from the National Cancer Institute, Canada.

[3]Division of Radiation Oncology, University of Alberta and Cross Cancer Institute, Edmonton, Canada.

[4]Department of Medicine, University of Alberta, Edmonton, Canada.

[5]Saunder MI, and Dische S. A report on skin reactions occurring in two patients on misonidazole. Personal communication, Oct 1977.

*_Reprint requests to:_ Dr. Raul C. Urtasun, Division of Radiation Oncology, The University of Alberta, 11560 University Ave, Edmonton, Alberta, T6G 1Z2 Canada.

ture rose to 38°C. The drug-related skin rash was less severe than the first onset, although there was the same degree of pruritus. As before, with resolution of the rash, petechiae appeared temporarily. The dermatitis resolved after 7 days.

Measurements of BUN, bilirubin, glucose, uric acid, alkaline phosphatase, SGOT, LDH, calcium, phosphorus, total protein, albumin, and creatinine were all normal. Wbc, hemoglobin, and platelet counts were within normal limits. At no time throughout and after the course of treatment were there signs of a developing peripheral neuropathy.

Patient 2

Patient 2 (a 64-year-old Caucasian male) was diagnosed as having a grade IV astrocytoma in the left parietal region. On March 15, 1978, a treatment plan of radiotherapy was initiated: 3900 rads in nine fractions three times a week for 3 weeks. Four and one-half hours prior to each treatment he received 2 g of misonidazole. There was a history of a sulfa drug allergy. Concomitant medications were dexamethasone, diphenylhydantoin, and prochlorperazine.

The first seven misonidazole doses were well-tolerated. Approximately 10 hours after receiving the eighth dose a maculopapular rash appeared on the perineum, later spreading to the thighs, abdomen, back, and arms. There was also a rash, although less intense, present on the palms. The patient remained afebrile with no complaints of itchiness. Diphenylhydantoin was discontinued. Forty-eight hours later the rash was beginning to disappear from the arms, but was more prominent on the back and abdomen. Seventy-two hours after developing the rash there were positive signs of its regression.

For therapeutic reasons and with the patient's consent, the final 2-g dose of misonidazole was administered. Dexamethasone was continued as before. Within 24 hours the erythematous maculopapular rash had become more prominent over the entire torso. The patient continued to be afebrile and had no pruritus. The dexamethasone was increased. With resolution of the rash, petechiae-like lesions developed on the legs and the abdomen. Seven days after drug ingestion there were no signs of a dermatitis.

Measurements of BUN, bilirubin, glucose, uric acid, alkaline phosphatase, SGOT, LDH, calcium, phosphorus, total protein, albumin, and creatinine, and wbc, hemoglobin, and platelet counts were within normal limits. There were no signs of a peripheral neuropathy.

TABLE 1.—Blood concentrations of misonidazole and its metabolite assessed by high-pressure liquid chromatography

	Concentrations (μM)	
	Misonidazole	Primary metabolite*
Patient 1		
1/16/78	(—)† 169‡	(—) 48
1/18/78	(7) 129	(—) 59
1/20/78	(7) 174	(—) 75
1/23/78	(30) 169	(—) 75
1/25/78	Sample missed	Sample missed
1/27/78	(—) 119	(—) 64
Patient 2		
3/15/78	(—) 119	(—) 53
3/17/78	(40) 109	(—) 48
3/20/78	(—) 109	(—) 40
3/22/78	(—) 99	(—) 43
3/27/78	(—) 99	(—) 35
3/29/78	(—) 99	(—) 32
3/31/78	(—) 119	(—) 37
4/3/78	(—) 154	(—) 32
4/6/78	(—) 109	(—) 32

*Hoffmann-La Roche Laboratories designation RO-05-9963.

†Nos. in parentheses = concentration of drug in control blood drawn just prior to next dose.

‡Concentration of drug 4½ hrs after drug ingestion.

DISCUSSION

Two patients receiving the hypoxic cell radiosensitizer, misonidazole, developed a disseminated erythematous maculopapular rash after being treated on a three times a week basis for 2½ weeks. One patient developed fever and severe pruritus, while the other patient was symptom-free. After concomitant medications were discontinued, except dexamethasone, both patients were given a challenge dose. Evidence is presented showing that in both cases the resulting dermatitis was related to the radiosensitizer, misonidazole. For both patients, serum concentrations of the drug remained within the expected ranges (table 1).

Previous incidences of various types of skin rashes have been reported for metronidazole, a 2-nitroimidazole derivative (5–9). Further occurrences of dermatitis may be expected with continued use of nitroimidazoles, such as misonidazole and metronidazole.

REFERENCES

1. URTASUN RC, BAND PR, CHAPMAN JD, ET AL . Clinical phase I study of the hypoxic cell radiosensitizer RO-07-0582, a 2-nitroimidazole derivative. Radiology 122:801–804, 1977.
2. TANNOCK I. Letter to the Editor: Metronidazole as a radiosensitizer. N Engl J Med 295:901–902, 1976.

3. DISCHE S, SAUNDERS MI, LEE ME, ET AL. Clinical testing of the radiosensitizer RO-07-0582: experience with multiple doses. Br J Cancer 35:567–579, 1977.

4. JENTZSCH K, KARCHER KH, KOGELNIK HD, ET AL. Initial clinical experience with the radiosensitizing nitroimidazole RO-07-0582. Strahlentherapie 153:825–831, 1977.

5. MAIZE J, and TOMECKI K. Letter: Pityriasis rosea-like drug eruption secondary to metronidazole. Arch Dermatol 113:1457–1458, 1977.

6. ROE FJC. Metronidazole: review of uses and toxicity. J Antimicrob Chemother 3:205–213, 1977.

7. RAO N, and REDDY V. Treatment of tropical pulmonary eosinophilia with metronidazole. J Trop Med Hyg 76:147–150, 1973.

8. NAIK RPC, and SINGH G. Fixed drug eruption due to metronidazole. Dermatologica 55:59–60, 1977.

9. GOODMAN LS, and GILLMAN A. The Pharmacological Basis of Therapeutics. New York, The MacMillan Co, 1975, pp 1086–1088.

Limited Activity of ICRF-159 in Advanced Acute Leukemia [1]

Brief Reports and Preliminary Communications

M. T. Bakowski [2,*] H. G. Prentice, [3,4] T. A. Lister, [2] J. S. Malpas, [2] T. J. McElwain, [3] and R. L. Powles [3]

SUMMARY

Twenty-eight patients with advanced acute nonlymphocytic leukemia, 16 with acute lymphoblastic leukemia, and two with acute undifferentiated leukemia were treated with ICRF-159. No patient achieved a complete remission and only three patients (6%) achieved a partial bone marrow remission. The only significant toxic effect was myelosuppression which probably contributed to the death of six patients. Five patients with acute nonlymphocytic leukemia and one with acute lymphoblastic leukemia received a combination of ICRF-159 and low-dose cytosine arabinoside. There were no remissions in this group and the toxic effects were more marked than with ICRF-159 alone. This study confirms the limited activity of ICRF-159 as a single agent in acute leukemia demonstrated in smaller series, and shows that, when used in combination with low-dose cytosine arabinoside, it was ineffective and resulted in increased toxicity. ICRF-159 alone or in combination with cytosine arabinoside has very limited activity in advanced adult acute leukemia.

[Cancer Treat Rep 63:127–129, 1979]

ICRF-159 is a bis-diketopiperazine derivative formed by the ring closure of ethylenediamine tetraacetic acid (EDTA). The drug was synthesized 12 years ago at the Imperial Cancer Research Fund in London and was released for clinical trial in the United Kingdom in 1969. Two reviews published recently cover the preclinical data and radiosensitizing properties of the drug (1) and the clinical data on the activity of the drug in solid tumors (2). There have been only limited studies of ICRF-159 in small series of patients with acute leukemia (3–6) (table 1). In this paper we report the results of the treatment with ICRF-159 as a single agent in 46 patients (43 adults and three children) with advanced acute leukemia refractory to conventional therapy. In view of the report by Hellmann's group (7) that the combination of ICRF-159 and low-dose cytosine arabinoside is effective and well-tolerated in acute nonlymphocytic leukemia (ANLL), we have also evaluated the activity of the combination in six patients.

MATERIALS AND METHODS

Twenty-eight patients with ANLL, 16 with acute lymphoblastic leukemia (ALL), and two with acute undifferentiated leukemia (AUL) received 183 courses (mean, four; median, two; and range, one to 35 courses) of ICRF-159 administered orally at doses ranging from 150 to 500 mg/m^2/day in divided doses every 8 hours for 3–5 days. Courses were repeated at 1–3-week intervals. The starting dose in most cases was about one third of the maximum-tolerated dose of 1 g/m^2/day for 3 days every 3 weeks established in phase I studies (8) in patients with normal marrow function. The dose was increased in 16 patients. The maximum dose administered in one course was 400 mg/m^2/day for 5 days.

Five patients with ANLL, including two who had received prior therapy with ICRF-159 alone, and one patient with ALL received 27 courses (mean and median, four; and range, one to 14 courses) of ICRF-159 (200–400 mg/m^2/day) in combination with cytosine arabinoside (35–70 mg/m^2/day) for 3 days repeated at 1–2-week intervals.

Evaluation during therapy included the monitoring of hematologic values, hepatic and renal function, and serum calcium levels. Partial remission (PR) of disease was defined as a decrease of $\geq 50\%$ in the bone marrow blast cell count.

[1]Received Aug 2, 1978; revised Oct 17, 1978; accepted Nov 27, 1978.

[2]Imperial Cancer Research Fund Department of Medical Oncology, St. Bartholomew's Hospital, London, England.

[3]The Leukaemia Unit, The Royal Marsden Hospital, Sutton, Surrey, England.

[4]*Present address:* Academic Department of Haematology, The Royal Free Hospital, London, England.

**Reprint requests to:* Dr. Marie T. Bakowski, Imperial Cancer Research Fund Department of Medical Oncology, St. Bartholomew's Hospital, West Smithfield, London EC1A 7BE, England.

TABLE 1.—Published data on ICRF-159 as a single agent in acute leukemia

No. of patients	ICRF-159 dose	No. of responses CR	No. of responses PR	Toxicity and comments	Reference No.
		ANLL			
3	8–43 mg/kg/day × 2–6 days	0	0	Uric acid nephropathy, alopecia, and vomiting	Hellmann et al (1969) (3)
4	20–30 mg/kg/day × 4 days	0	0	Leukopenia and alopecia	Hellmann (1970) (4)
3	300 mg/m²/day	0	0	Leukopenia and alopecia	Mathé et al (1970) (5)
2	20 mg/kg/day	0	1	Leukopenia; PR for 2 wks	Krepler et al (1975) (6)
1	300 mg/m²/day × 3 days	0	0		
13		0	1 (8%)		
		ALL			
2	13–31 mg/kg/day × 2–12 days	0	1	Neutropenia and vomiting; PR for 3+ wks	Hellmann et al (1969) (3)
3	20–30 mg/kg/day × 4 days	0	0	Leukopenia and alopecia	Hellmann (1970) (4)
7	300 mg/m²/day	2	0	Neutropenia and thrombocytopenia	Mathé et al (1970) (5)
6	20 mg/kg/day × 4 days	0	2	Leukopenia; PR for 10 days and 2 wks	Krepler et al (1975) (6)
4	300 mg/m²/day × 3 days	1	0	CR for 4 mos	
22		3 (14%)	3 (14%)		

RESULTS

Forty-five patients were evaluable for response to ICRF-159 as a single agent. No patient achieved a complete remission (CR). One adult with ANLL, one with ALL, and one with AUL achieved a bone marrow PR lasting for 8, 12, and 46+ weeks, respectively. None of the six patients treated with ICRF-159 in combination with cytosine arabinoside achieved a remission.

The major toxic effect observed was myelosuppression which was seen at all dose levels and did not appear to correlate with either the schedule of administration or the number of courses administered. Of 46 evaluable patients, 28 (60%) developed neutropenia (decrease of blood cell count to $< 1000/mm^3$ or $> 50\%$ reduction) and 14 (30%) developed thrombocytopenia (decrease of blood cell count to $< 100{,}000/mm^3$ or $> 50\%$ reduction). Both neutropenia and thrombocytopenia were seen in six patients, five of whom had ALL. Overall, patients with ALL experienced more toxic effects; neutropenia occurred in 75% of ALL patients as compared with 57% of ANLL patients, and thrombocytopenia occurred in 62% of ALL patients as compared with only 14% of ANLL patients. Six deaths from hemorrhage were associated with drug-induced thrombocytopenia. No deaths could be directly attributed to drug-induced neutropenia. Toxic effects apart from myelosuppression were minimal. Two children experienced nausea with their first course of treatment. There was no evidence of renal or hepatic damage, and there were no reports of alopecia.

Myelosuppression was more marked in the six patients treated with the combination of ICRF-159 and cytosine arabinoside. All patients developed neutropenia and five developed thrombocytopenia. One patient who had experienced only neutropenia when treated with ICRF-159 alone, developed both neutropenia and thrombocytopenia and a syndrome of fever, malaise, and vomiting, lasting from Day 3 to Day 5 of each course of the combination; he also reported hair loss.

DISCUSSION

No CRs and only three (6%) bone marrow PRs were seen in 45 patients with acute leukemia treated with ICRF-159 as a single agent. These results are unimpressive, even taking into account that the patients were in an advanced state of disease refractory to conventional therapy. Myelosuppression was severe in many cases and probably contributed to six deaths from hemorrhage. Combin-

ing ICRF-159 with low-dose cytosine arabinoside did not improve the remission rate and merely increased the toxic manifestations. The results of this study show that ICRF-159 used as a single agent or in combination with low-dose cytosine arabinoside has very limited activity in advanced adult acute leukemia. We suggest that ICRF-159 is not sufficiently active in the doses and schedules tested to warrant further evaluation in adult acute leukemia.

REFERENCES

1. BAKOWSKI MT. ICRF 159, (±) 1,2-di(3,5-dioxopiperazin-1-yl)-propane NSC-129,943; Razoxane. Cancer Treat Rev 3:95–107, 1976.
2. BELLET RE, ROZENCWEIG M, VON HOFF DD, ET AL. ICRF-159: current status and clinical prospects. Eur J Cancer 13:1293–1298, 1977.
3. HELLMANN KA, NEWTON KA, WHITMORE DN, ET AL. Preliminary clinical assessment of ICRF 159 in acute leukaemia and lymphosarcoma. Br J Med 1:822–824, 1969.
4. HELLMANN K. Further clinical experiences with ICRF 159. *In* Recent Results in Cancer Research (Mathé G, ed). New York, Springer-Verlag, 1970, pp 52–53.
5. MATHÉ G, AMIEL JL, HAYAT M, ET AL. Preliminary data on acute leukemia treatment with ICRF 159. *In* Recent Results in Cancer Research (Mathé G, ed). New York, Springer-Verlag, 1970, pp 54–55.
6. KREPLER P, and PAWLOWSKY J. Clinical trials with bis-dioxopiperazine propane (ICRF 159; NSC 129,943) in acute leukaemias. Osterr Z Onkol 2:112–114, 1975.
7. HELLMANN K, NEWTON KA, HUMBLE JG, ET AL. Razoxane and cytosine arabinoside: an alternative first-line combination for remission induction in acute non-lymphocytic leukaemia. *In* Current Chemotherapy, Proceedings of the 10th International Congress of Chemotherapy, Zurich, Sept 18–23, 1977. Washington, DC, American Society for Microbiology, 1978, pp 1178–1180.
8. BELLET RE, MASTRANGELO MJ, DIXON LM, ET AL. Phase I study of ICRF-159 (NSC-129943) in human solid tumors. Cancer Chemother Rep 57:185–189, 1973.

Isotopic Immunoglobulin in an Integrated Multimodal Treatment Program for a Primary Liver Cancer: A Case Report [1,2]

Brief Reports and Preliminary Communications

David S. Ettinger, Leon H. Dragon, Jerry Klein, Maggie Sgagias, and Stanley E. Order [3,*]

SUMMARY

An intrahepatic cholangiocarcinoma was treated with a new multimodality treatment program which integrated low-dose adriamycin, 5-fluorouracil, the radiation sensitizer metronidazole, and liver irradiation (1750 rads) followed by iv administration of 47 millicuries of ^{131}I-labeled carcinoembryonic antibody. A computerized axial tomographic scan taken after the external irradiation and drug combinations was unchanged from the pretreatment status. Following isotopic immunoglobulin treatment, significant clinical remission was documented by physical examination and a repeat computerized axial tomographic scan. During isotopic immunoglobulin treatment, sequential whole-body isotope scans revealed tumor localization and isotope persistence. This case report is reviewed because of the unique therapeutic regimen and the possibility of future exploration of isotopic immunotherapy in radiation oncology.

[Cancer Treat Rep 63:131–134, 1979]

Surgery remains the treatment of choice in primary cancer of the liver. Conventional treatment with radiation therapy and/or systemic chemotherapy has not produced any appreciable effect on survival rates (1–5). The presentation of a 38-year-old woman with a nonresectable, extensive intrahepatic biliary adenocarcinoma led to an extensive search for a multimodality approach for therapeutic management.

The restrictions of a case report and of any broader conclusions are fully realized. However, this case is reported because of the use of new multimodality therapy and the many interesting facets of therapeutic application in association with the remission obtained.

MATERIALS AND METHODS

Purified carcinoembryonic antigen (CEA) was kindly supplied by Drs. P. Gold and M. J. Krantz (McGill University, Montreal, Quebec, Canada). Antiserum preparation followed standard methodology using New Zealand white rabbits (6). Radiolabeling was carried out by the lactoperoxidase technique (7). The reactivity of the radiolabeled IgG was confirmed by immunoelectrophoresis. Three samples of antibody were pyrogen-tested at an independent research laboratory prior to clinical use.

CASE REPORT

A 38-year-old white woman was in good health until September 1977 when she developed anorexia, weight loss, and abdominal pain. Continued symptoms led to admission to The Johns Hopkins Hospital in January 1978. Physical examination was normal except for abdominal tenderness in the epigastrium and right upper quadrant and an enlarged liver three fingerbreadths below the right costal margin.

Laboratory studies were normal except for a hematocrit of 32%; serum calcium of 12 mg/dl (normal, 9–11 mg/dl); alkaline phosphatase of 53 IU/liter (normal, 10–32 IU/liter); and CEA of 4.8 ng/ml. A liver scan, sonogram, and computerized axial tomographic (CAT) scan revealed a large mass in the right lobe of the liver (fig 1). A laparotomy on January 20, 1978, revealed a nonresectable adeno-

[1]Received Aug 21, 1978; revised Nov 9, 1978; accepted Nov 20, 1978.

[2]Supported in part by Public Health Service grant CA-06973 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare, and by the Stuart and Barbara Janney Fund.

[3]Sections of Medical Oncology (*D. S. Ettinger* and *L. H. Dragon*) and Radiation Oncology (*J. Klein, M. Sgagias,* and *S. E. Order*), Comprehensive Cancer Center, The Johns Hopkins Hospital, Baltimore, Md.

**Reprint requests to:* Dr. Stanley E. Order, Johns Hopkins Oncology Center, The Johns Hopkins Hospital, Baltimore, Md 21205.

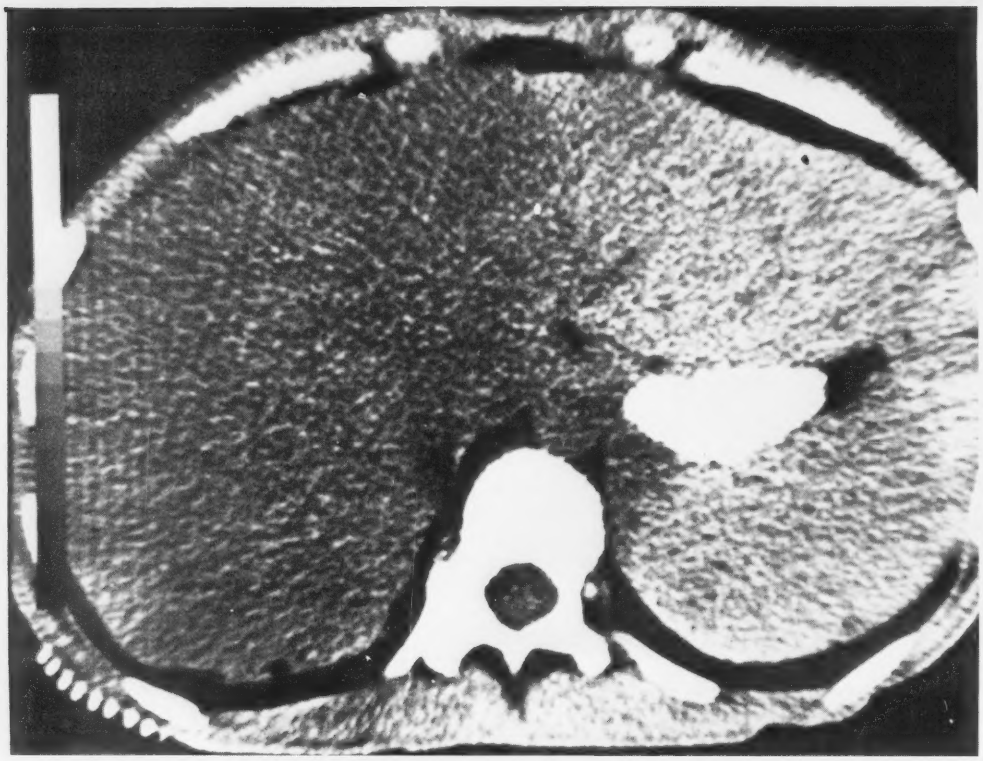


FIGURE 1.—CAT scan prior to isotopic immunotherapy demonstrating the enlarged liver and massive tumor (dark region) in the right lobe and extending across the midline.

carcinoma consistent with primary cholangiocarcinoma of the liver.

On February 3, 1978, following informed consent, the patient began to receive radiation therapy to the liver, 250 rads/day × 7 days. Four hours prior to the radiation therapy the patient received 9 g of metronidazole (Flagyl) orally on Days 1, 3, 5, and 7. On Days 2, 4, and 6, 2 hours prior to the radiation therapy, she received 15 mg of adriamycin iv and 500 mg of 5-fluorouracil (5-FU) iv. She experienced no significant side effects. During treatment the patient lost 4.0 kg of weight and therefore received 1 week of peripheral iv hyperalimentation, regaining 2 kg of weight. A repeat CAT scan showed no change in status. Five weeks after completion of the radiation therapy, the patient received monthly courses of combination chemotherapy with adriamycin at a dose of 50 mg/m^2 iv and 5-FU at a dose of 600 mg/m^2 iv. A repeat CAT scan was unchanged.

In April the patient was skin and eye tested for rabbit IgG and was not allergic. Lugol's solution was taken daily for several days prior to the iv administration of 47 millicuries of ^{131}I-labeled CEA antibody. Following administration, whole-body scans were taken daily, and at 48 hours a clearly demarcated tumor in the liver was noted (fig 2). Several days later, ^{131}I-labeled IgG uptake occurred in the thyroid gland. The patient's thyroid stimulating hormone level was followed and remains normal. An additional CAT scan with contrast revealed significant reduction in tumor (fig 3). Liver size had returned to normal and chemistry studies remain normal 8 months after initiation of therapy.

DISCUSSION

Experimental and clinical scans for carcinoma should be done several days after injection of radiolabeled antibody to visualize the tumor with tumor:muscle ratios of 6–8 to 1 (8–11).

Present clinical exploration in the application of anti-CEA antibody has indicated that at least 20% of the antibody is deposited in the tumor-bearing region. However, the liver receives more irradiation

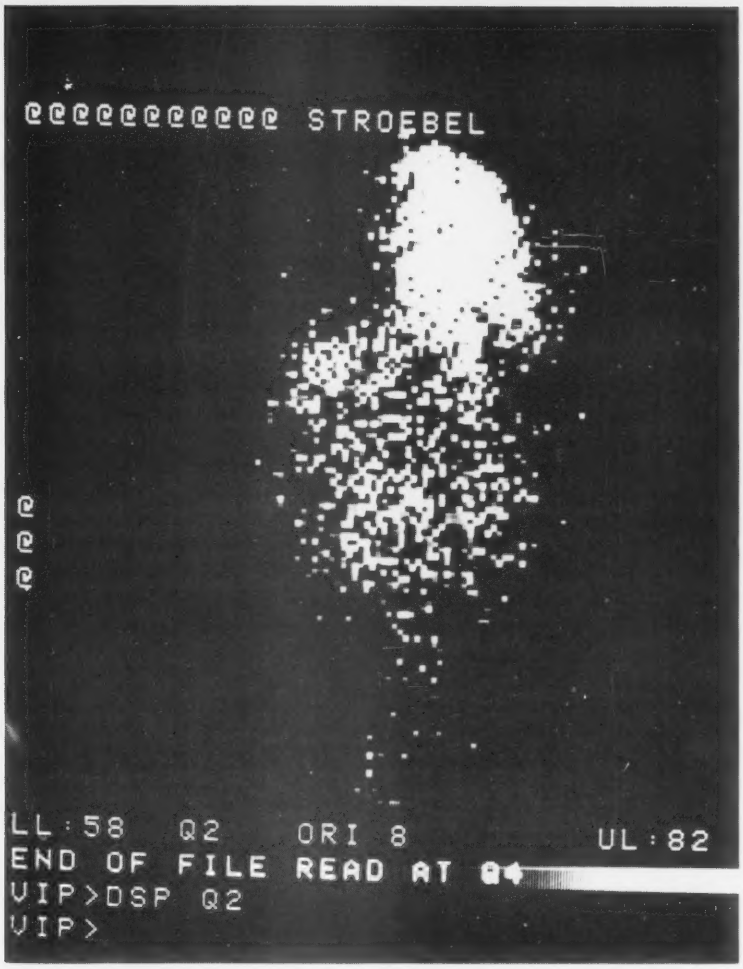


FIGURE 2.—Radioimmunoglobulin scan (vertical) after iv administration of ^{131}I-labeled CEA antibody demonstrating the cardiac region (due to isotope in the blood) and the liver tumor (48 hrs after treatment).

due to nonspecific binding that occurs with foreign antibody.

In the present case, blood level determinations indicated CEA elaboration (4.8 ng/ml) and reduction after antiserum administration (2.4 and 2.9 ng/ml). Direct tissue evidence of CEA activity was not obtained.

The doses of ^{131}I administered have been in the range used for the treatment of thyroid cancer, ie, 50–125 millicuries. Persistence of radiolabeled antibody in the tumor-bearing site has been noted for 8 days. The only organ that would be restrictive in exposures at this level of irradiation would be the bone marrow, since the potential total-body dose of irradiation is presently in the range of 50–200 rads. Previous chemotherapy and irradiation might further limit such treatment due to previous hematopoietic depression. We have not observed complications with regard to antigen-antibody complexes in >25 administrations of heteroimmune tumor antisera. However, a major limitation in repeated applications is immune reactivity to foreign antibody. This may be avoided by administration of foreign immunoglobulin within the first week of exposure or by changing the species to avoid host sensitization. Of course, immune sensitization may have led

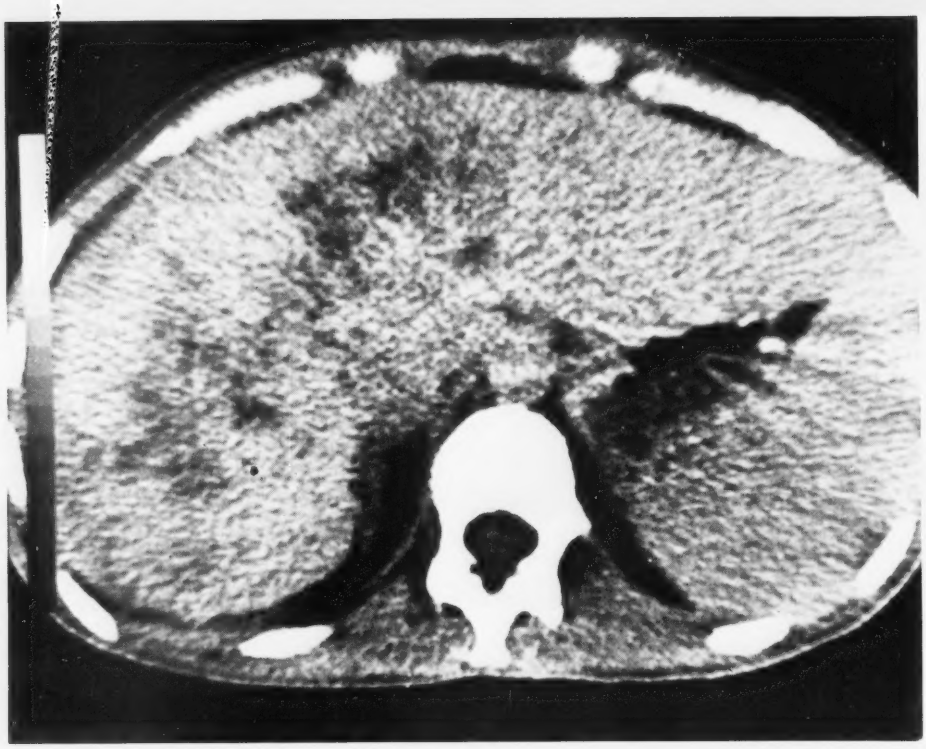


FIGURE 3.—Posttreatment CAT scan demonstrating resolution of a significant proportion of the tumor (dark region). CAT scan was carried out in the same plane as the pretreatment scan. Physical examination at this time revealed a normal-sized liver.

to the therapeutic effectiveness in the present clinical response and this has been described in an experimental model (12).

At present, even though a method of estimation of dose is available, we do not have correlative data to determine the precision of such estimates and prefer to report on the administered dose. The need for quantitation in future studies is recognized. The integration of isotopic immunoglobulin into treatment protocols offers many new possibilities. Hepatic malignancy may be particularly ideal for isotopic immunoglobulin therapy because of both specific and nonspecific antibody binding.

REFERENCES

1. CRUTCHAT HP, TERZ JJ, KAY S, ET AL. Primary liver cancer. Surgery 70:467–479, 1971.
2. EL-DOMEIRI AA, HIVOS AG, GOLDSMITH HS, ET AL. Primary malignant tumors of the liver. Cancer 27:7–11, 1971.
3. ONG GB, and CHAN PK. Primary carcinoma of the liver. Surg Gynecol Obstet 143:31–38, 1976.
4. CHAN KT. The management of primary liver carcinoma. Ann R Coll Surg Engl 41:253, 1971.
5. OLWENY CLM, TOYA T, KATONGOLE-MBIDDE E, ET AL. Preliminary communication: Treatment of hepatocellular carcinoma with adriamycin. Cancer 36:1250–1257, 1975.
6. WRIGHT T, SINANAN M, HARRINGTON D, ET AL. Immunoglobulin: applications to scanning and treatment. Appl Radiol. In press.
7. MARCHILONIS JJ. An enzymatic method for the trace iodination of immunoglobulins and other proteins. Biochem J 113:299–305, 1969.
8. ORDER SE. The history and progress of serologic immunotherapy and radiodiagnosis. Radiology 118:219–223, 1976.
9. GOLDENBERG DM, DELAND F, KIM E, ET AL. Use of radiolabelled antibodies to carcinoembryonic antigen for the detection and localization of diverse cancers by external photoscanning. N Engl J Med 298:1384–1388, 1978.
10. HOFFER PB, LATHROP K, BERKERMAN C, ET AL. Use of I^{131} CEA antibody as a tumor scanning agent. J Nucl Med 15:323–327, 1974.
11. MACH JP, CARREL S, MERENDA C, ET AL. In vivo localization of radiolabelled antibodies to carcinoembryonic antigen human colon carcinoma grafted into nude mice. Nature 248:704–706, 1974.
12. ORDER SE, KIRKMAN R, and KNAPP R. Serologic immunotherapy: results and probable mechanism of action. Ca 34:163–171, 1974.

Rubidazone in Metastatic Breast Cancer [1,2]

Sewa S. Legha,* Robert S. Benjamin, Aman U. Buzdar, G. N. Hortobagyi, and George R. Blumenschein [3]

Brief Reports and Preliminary Communications

Rubidazone is a semisynthetic daunorubicin derivative that has striking antitumor activity in acute leukemia (1–3). Because the closely related anthracycline adriamycin is the most active single agent in the treatment of metastatic breast cancer, it was of interest to determine the antitumor activity of rubidazone against this tumor.

PATIENTS AND METHODS

Rubidazone was supplied by the National Cancer Institute, Bethesda, Md. Based on our experience in a phase I study of rubidazone in patients with solid tumors, we chose an initial rubidazone dose of 150 mg/m² for breast cancer patients, since they tend to have somewhat more severe myelosuppression than patients with other solid tumors for whom a dose of 180–200 mg/m² is recommended. If the serum bilirubin level was elevated, rubidazone was reduced by 25%–50%. All patients were premedicated with 50 mg of Benadryl iv. Rubidazone was dissolved in 250–500 ml of 5% dextrose in water and infused over a period of 30 minutes to 1 hour. Treatment was repeated at 3-week intervals. The investigational nature of this study was explained to the patients and an informed consent was obtained before the drug was administered.

All patients entered in this study had histologic diagnosis of metastatic breast cancer and had received prior treatment with chemotherapy, most commonly with a combination of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). No patient with prior treatment and demonstrated refractoriness to adriamycin was eligible for this study. All patients were postmenopausal and most had received prior endocrine therapy. In all cases serum creatinine and serum bilirubin levels were < 2 mg/dl. All of the patients had measurable disease and an expected survival of ≥ 2 months, with a performance score (Eastern Cooperative Oncology Group) of ≥ 3. The definition of an objective clinical response was standard: a $\geq 50\%$ decrease in the product of the perpendicular diameter of all measurable disease without evidence of new lesions or an increase of $> 25\%$ of any lesions.

A total of 14 patients were entered in this protocol. One patient was not evaluable because she received rubidazone and DTIC simultaneously. The general characteristics of the 13 evaluable patients are summarized in table 1. One patient had received two doses of adriamycin in another institution prior to receiving rubidazone in our clinic. She was considered evaluable in this protocol because it was not certain whether she was refractory to adriamycin or not. Most of the patients were refractory to CMF or CMF and prednisone therapy used either as adjuvant therapy or after development of metastatic disease. Most patients had extensive metastatic disease and ten of the 13 patients had visceral metastases.

RESULTS

The antitumor activity of rubidazone was disappointing. None of the patients showed objective tumor regression. One patient had stabilization of disease for 5 months and received a total of seven courses of rubidazone before her disease progressed. Two patients received a single dose of rubidazone and died within 1 month of starting therapy. Most of the other patients received two to three courses of treatment before the disease progressed.

Toxicity of rubidazone was modest. Gastrointestinal toxicity was insignificant and consisted of mild nausea and occasional vomiting. Although rubidazone commonly caused chills and fever in our initial phase I study in acute leukemia, premedication with an antihistamine completely ameliorated these effects and only one instance of a fever of 101°F was observed. The most common toxicity was myelo-

[1]Received Aug 28, 1978; revised Nov 13, 1978; accepted Nov 28, 1978.

[2]Supported in part by contract N01-CM-43801 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Departments of Developmental Therapeutics (*S. S. Legha* and *R. S. Benjamin*) and Medicine (*A. U. Buzdar, G. N. Hortobagyi,* and *G. R. Blumenschein*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Sewa S. Legha, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, Tex 77030.

TABLE 1.—Characteristics of rubidazone-treated patients

Patient No.	Age	Extent of disease	Prior chemotherapy	Rubidazone therapy: Date started (mo/yr)	Rubidazone therapy: Response	Comments (mo/yr)
1	43	Bones	CMF	8/77	Progression after 3 doses	Alive 7/78
2	58	Bones, liver	CMF + prednisone, melphalan	10/77	Progression after 2 doses	Died 2/78
3	52	Skin, lung	CMF, adriamycin*	6/77	Progression after 3 doses	Alive 5/78
4	58	Bones, liver	CMF + vincristine and prednisone	9/76	Progression after 2 doses	Died 8/77
5	56	Bones, pleura	CMF	5/77	Stable for 5 mos (7 doses)	Alive 6/78
6	34	Bones, lung	CMF	7/77	Progression after 1 dose	Died 8/77
7	50	Bones, lung	CMF	11/77	Progression after 4 doses	Alive 8/78
8	49	Bones, skin	CMF + prednisone	8/77	Progression after 3 doses	Alive 1/78
9	67	Bones	CFP	8/77	Progression after 2 doses	Alive 7/78
10	67	Bones, liver	CFP	7/77	Progression after 3 doses	Died 2/78
11	59	Skin, lung, pleura, bones	CMF	10/77	Progression after 2 doses	Alive 7/78
12	52	Liver	CMF	11/77	Progression after 1 dose	Died 12/77
13	64	Bones, liver	CMF	12/76	Progression after 2 doses	Died 3/77

*This patient had stable disease after receiving 2 cycles of adriamycin.

suppression. In patients who had tumor in the bone marrow or extensive liver metastases the degree of myelosuppression was more marked. Of the four patients with extensive liver metastases who had normal serum bilirubin levels and were receiving full doses of rubidazone, the median lowest granulocyte count was 600/mm^3 and the median lowest platelet count was 48,000/mm^3. In three patients who had extensive bone disease with replacement of bone marrow by tumor, the median lowest granulocyte count was 600/mm^3 and the median lowest platelet count was 87,000/mm^3. In patients without extensive liver or bone disease the lowest granulocyte count was 1300/mm^3 and the platelet counts were normal. Based on these data it appears that the rubidazone dose of 150 mg/m^2 as used in this study was appropriate for most of the patients.

Although daunorubicin is one of the most active single drugs in acute leukemia, its antitumor activity against solid tumors has not been adequately evaluated (4). Similarly, rubidazone has remarkable antileukemic activity, but its usefulness in solid tumors other than breast cancer has not been determined. In metastatic breast cancer, rubidazone appears to be clearly inferior to adriamycin, which has resulted in response rates of 20%–35% in previously treated breast cancer patients (5). Because of a lack of any response in 13 consecutive patients, we felt it was unjustified to continue this trial any further. Using Gehan's principle of determining the sample size of patients required to judge the therapeutic efficacy of a phase II agent, our data indicate that there is a $> 90\%$ probability that the response rate of metastatic breast cancer to rubidazone would be $< 20\%$ (6), suggesting that adriamycin has superior activity in this disease.

REFERENCES

1. JACQUILLAT CL, WEIL M, GEMON MF, ET AL. Clinical study of rubidazone (22050R.P.), a new daunorubicin-derived compound in 170 patients with acute leukemias and other malignancies. Cancer 37:653–659, 1976.
2. BENJAMIN RS, KEATING MJ, MCCREDIE KB, ET AL. A phase I and II trial of rubidazone in patients with acute leukemia. Cancer Res 37:4623–4628, 1977.
3. BENJAMIN RS, KEATING MJ, SWENERTON KN, ET AL. Clinical trials of rubidazone alone and in combination in patients with acute leukemia and solid tumors. *In* Current Chemotherapy (Proceedings of the 10th International Congress of Chemotherapy, Zurich, Switzerland, Sept 18, 1977) (Siegenthaler W, and Lüthy R, eds). Washington, DC, American Society of Microbiology, 1978, vol 2, pp 1246–1248.
4. VON HOFF DD, ROZENCWEIG M, SLAVIK M, ET AL. Activity of daunomycin in solid tumors. JAMA 236:1693, 1976.
5. FREDERIKSEN PL, JOERGENSEN ST, ROESDAHL K, ET AL. Activity of adriamycin in metastatic breast cancer resistant to a combination regimen with cyclophosphamide, methotrexate, 5-fluorouracil, vincristine, and prednisone. Cancer Treat Rep 62:449–450, 1978.
6. GEHAN EA. The determination of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. J Chronic Dis 13:346–353, 1961.

Phase II Trial of Hexamethylmelamine in Ovarian Carcinoma Resistant to Alkylating Agents [1]

Brief Reports and Preliminary Communications

Philip D. Bonomi,* John Mladineo, Bonnie Morrin, George Wilbanks, Jr, and Robert E. Slayton [2]

Previous phase II trials have demonstrated that hexamethylmelamine (HMM) is active in ovarian adenocarcinoma (1–3). In one of these trials, responses were observed in three patients who had tumors which were resistant to alkylating agents (3). The present study was designed to test the efficacy of HMM in alkylating agent-resistant ovarian carcinoma. HMM was given daily because recent laboratory data suggest that daily administration increases HMM's effectiveness (4).

PATIENT SELECTION

To be eligible patients had to have measurable, histologically confirmed stage III or IV epithelial ovarian carcinoma which had progressed during treatment with an alkylating agent. Recovery from the effects of previous chemotherapy or radiotherapy was required. Additional eligibility requirements were: wbc count $> 4000/mm^3$, platelet count $> 100,000/mm^3$, BUN < 25 mg/dl, serum creatinine < 1.5 mg/dl, bilirubin < 1.5 mg/dl, and SGOT < 60 IU/liter. Patients who were bedridden (Eastern Cooperative Oncology Group performance status, 4) were excluded.

DRUG ADMINISTRATION

HMM was supplied by the National Cancer Institute, Bethesda, Md, in 50- and 100-mg capsules. The dose was 250 mg/m^2/day in four divided doses. Body surface area was calculated from the lesser of actual or ideal body weight. HMM was continued until there was evidence of tumor progression or significant toxicity.

DOSE MODIFICATIONS

The dose of HMM was decreased by 50% for the following reasons: moderate nausea and vomiting, leukocyte count between 3000 and 4000/mm^3, and mild dystaxia. The drug was discontinued in the following instances: leukocyte count $< 3000/mm^3$, platelet count $< 100,000/mm^3$, severe nausea and vomiting, or disabling paresthesias and/or dystaxia.

EVALUATION

Tumor responses were defined by the following criteria: *Complete remission (CR)*—the complete disappearance of all pretherapy lesions with no new lesions appearing for at least 1 month; *partial remission (PR)*—a $> 50\%$ reduction in the sum of the products of the perpendicular diameters of all measurable lesions for at least 1 month; *stable disease (SD)*—a $< 50\%$ reduction or a $< 25\%$ enlargement of measurable lesions lasting at least 2 months; and *progression*—the appearance of new lesions or a 25% increase in pre-existing lesions.

RESULTS

Nineteen patients were entered in the study. All patients were evaluable for toxicity and 16 were evaluable for tumor response. Patients unevaluable for tumor response included two who were unable to tolerate HMM because of nausea and vomiting and one who had not been treated previously with an alkylating agent. Seventeen patients received HMM for 1–24 months with a median of 7 months in patients who had a response or SD.

Type and duration of tumor responses and duration of survival are shown in table 1. One CR and

TABLE 1.—Responses

Type	No.	Duration (mos)	Survival (mos)
CR	1	25+	27+
PR	3	5,12,14	15,18,23
SD	6	4,5,6,7,9,10+	7,9,9,10+,13,15
Progression	6	—	2,2,4,6,11,16+

[1]Received Aug 31, 1978; revised Nov 15, 1978; accepted Nov 28, 1978.

[2]Departments of Internal Medicine (P. D. Bonomi, B. Morrin, and R. E. Slayton) and Obstetrics and Gynecology (J. Mladineo and G. Wilbanks, Jr), Rush-Presbyterian-St. Luke's Medical Center, Chicago, Ill.

**Reprint requests to:* Philip D. Bonomi, MD, Section of Medical Oncology, Department of Internal Medicine, Rush-Presbyterian-St. Luke's Medical Center, 1753 W Congress Pkwy, Chicago, Ill 60612.

three PRs were observed 6–12 weeks after HMM was started. The CR was documented by repeat laparotomy, and this patient remains in CR 17 months after HMM was stopped. In each of the four responding patients regression involved a pelvic mass, and in one of the patients a supraclavicular mass also regressed. PRs occurred despite dose reductions of 50% in two patients and a dose reduction of 25% in a third patient. Nausea and vomiting occurred in seven patients and was eliminated in five patients by reducing the dose. Two patients continued to have nausea and vomiting and were dropped from the study. Leukopenia and/or thrombocytopenia requiring dose modifications occurred in six patients. Of seven instances of decreased blood cell counts, five occurred 6–8 weeks after starting HMM and two occurred 6 and 7 months later. Counts returned to normal 2–3 weeks after stopping HMM. The lowest wbc count was 1900/mm^3 and the lowest platelet count was 48,000/mm^3.

Three patients noted paresthesias in their hands and feet, and two patients experienced ataxia. These symptoms occurred 4–17 months after starting HMM. Ataxia cleared 1–2 weeks after stopping HMM and did not reappear when the drug was restarted at 50% of the previous dose.

DISCUSSION

Classic alkylating agents are the most active single drugs in ovarian carcinoma, with response rates ranging from 40% to 50% (5,6).In a randomized prospective trial in previously untreated patients, Smith and Rutledge (7) reported HMM more active than either melphalan or 5-fluorouracil. In addition, Young (8) and Wampler et al (3) have collectively reported eight responses among 20 patients with ovarian carcinoma resistant to alkylating agents. The four responses observed in our 16 patients confirm these earlier reports indicating a lack of cross-resistance between HMM and alkylating agents in some patients with ovarian carcinoma.

REFERENCES

1. WILSON WL, SCHROEDER JM, BISEL HF, ET AL. Phase II study of hexamethylmelamine (NSC 13875). Cancer 23:132–136, 1969.
2. WILSON WL, BISEL HF, COLE D, ET AL. Prolonged low-dosage administration of hexamethylmelamine (NSC 13875). Cancer 25:568–570, 1970.
3. WAMPLER GL, MELLETTE SJ, KUPERMINC M, ET AL. Hexamethylmelamine (NSC-13875) in the treatment of advanced cancer. Cancer Chemother Rep 56:505–514, 1972.
4. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine. An evaluation of its role in the therapy of cancer. Cancer 38:27–35, 1976.
5. TOBIAS JS, and GRIFFITHS CT. Management of ovarian carcinoma: current concepts and future prospects. Part 1. N Engl J Med 294:818–823, 1976.
6. ————. Management of ovarian carcinoma: current concepts and future prospects. Part 2. N Engl J Med 294:877–882, 1976.
7. SMITH JP, and RUTLEDGE FN. Random study of hexamethylmelamine, 5-fluorouracil, and melphalan in treatment of advanced carcinoma of the ovary. Natl Cancer Inst Monogr 42:169–172, 1975.
8. YOUNG RC. Chemotherapy of ovarian carcinoma: past and present. Semin Oncol 2:267–276, 1975.

Phase II Evaluation of Pyrazofurin in Patients With Advanced Colorectal Carcinoma [1,2,3]

Brief Reports and Preliminary Communications

David S. Carroll, Nancy E. Kemeny, and Richard J. Gralla [4,5,*]

Pyrazofurin (PZF) is an antibiotic product of *Streptomyces candidus*. After conversion to the monophosphate, PZF blocks pyrimidine biosynthesis by inhibiting orotidylate decarboxylase. PZF has been reported to be active against several animal tumor models including Walker 256 carcinosarcoma and 755 adenocarcinoma (1,2). In early clinical trials responses were seen in occasional patients with acute myelogenous leukemia, mycosis fungoides, and breast carcinoma (2,3). Significant activity was not observed in completed phase II studies when the drug was administered weekly to patients with lung cancer, sarcoma, or melanoma (4–6). A trial of PZF given every 3–4 weeks at a dose of 240 mg/m^2 to 35 patients with colorectal carcinoma demonstrated mild toxicity but no objective responses (7). Because of the observation that PZF inhibits > 95% of orotidylic acid metabolism 24 hours following an iv dose, but that it drops to 30% inhibition by 9 days (3), weekly doses of PZF were administered to patients with advanced colorectal carcinoma at doses which had shown manageable toxicity.

MATERIALS AND METHODS

Between July 1976 and July 1978, 26 patients with histologically confirmed metastatic colorectal adenocarcinoma and measurable disease participated in the study. An adequate drug trial was defined as a > 12-mg/kg total dose and at least three weekly doses. The characteristics of the 21 adequately treated patients are presented in table 1. A partial remission was defined as a ≥ 50% decrease of all measurable disease for at least 1 month. A minor response was defined as an objective tumor decrease (≥ 25%), but less than that required for a partial remission. Seven patients had one known area of metastatic disease while all others had multiple metastatic sites.

PZF was administered as a rapid iv injection. Initial doses were 4–5 mg/kg (160–200 mg/m^2). Doses

TABLE 1.—Characteristics and toxic effects of 21 patients treated with pyrazofurin

Characteristic	No. of patients
Age	
Range: 35–79 yrs	
Median: 63 yrs	
Performance status	
Range: 50–100	
Median: 70	
Prior treatment	
Chemotherapy only	13
Chemotherapy and radiation therapy	8
Sites of measurable disease	
Lung	13
Liver	12
Lymph nodes	4
Skin	1
Vagina	1
Toxic effects	
Mucositis	9
Nausea and vomiting	5
Wbc count nadir (cells/mm^3)	
≥ 4000	10
3000–3999	8
2000–2999	2
1000–1999	1
Platelet count nadir (cells/mm^3)	
≥ 150,000	6
100,000–149,999	8
50,000–99,999	4
< 50,000	3
Hemoglobin decrease (g/100 ml)	
0–1.9	3
2.0–2.9	5
3.0–3.9	7
≥ 4.0	6

[1]Received Aug 31, 1978; accepted Oct 31, 1978.

[2]Supported in part by contract N01-CM-57043 (Division of Cancer Treatment) and Public Health Service grants CA-05826 and CA-15928 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3]Pyrazofurin was supplied by Eli Lilly and Co, Indianapolis, Ind.

[4]Clinical Chemotherapy Service (*R. J. Gralla*) and Solid Tumor Service (*N. E. Kemeny*), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY. D. S. Carroll is a Fellow in Medical Oncology.

[5]We wish to thank Kathleen Trainor and Susan Andersen for preparation of the manuscript.

*Reprint requests to: Dr. Richard J. Gralla, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

were increased by 0.5 mg/kg/week (20 mg/m^2) in the absence of definite toxicity and were attenuated or withheld for moderate mucositis or blood cell count depression, as has been reported in prior PZF trials (4,5).

RESULTS

No objective responses occurred. Fourteen patients developed progressive disease while receiving PZF and seven others showed no change after a median of six doses. Total doses of PZF ranged from 12 to 81 mg/kg with a median total dose of 24 mg/kg.

The toxic effects of PZF therapy are also shown in table 1. As noted in several previous phase II trials, a normochromic normocytic anemia was the most frequently encountered toxic and dose-limiting effect. When a drop in the hemoglobin level of $\geq$ 2 g/100 ml occurred, chemotherapy was withheld and recovery was usually observed within 2 weeks. The median fall in hemoglobin was 3.2 g/100 ml. Mucositis was seen in nine adequately treated patients.

DISCUSSION

Creagan et al (7) did not detect responses in 35 patients treated every 3–4 weeks. Despite weekly administration during which nearly three times as much PZF was administered per treatment period as in the previously mentioned study, we also did not observe antitumor activity in our 21 adequately treated patients. Although all patients who participated in this study had received previous chemotherapy, their general condition was good and permitted outpatient management in most cases. It can be concluded that PZF administered weekly at a dose of 5 mg/kg (200 mg/m^2) is likely to have a $< 15\%$ true response rate ($P = 0.05$) (8) as a single agent in patients with previously treated colorectal adenocarcinoma.

REFERENCES

1. SWEENEY MJ, DAVIS FA, GUTOWSKI GE, ET AL. Experimental antitumor activity of pyrazomycin. Cancer Res 33:2619–2623, 1973.
2. GUTOWSKI GE, SWEENEY MJ, DELONG DC, ET AL. Biochemistry and biological effects of the pyrazofurins (pyrazomycins): initial clinical trial. Ann NY Acad Sci 255:544–551, 1975.
3. CADMAN EC, DIX DE, HILL SC, ET AL. Pyrazofurin: biochemical and clinicopharmacological studies. Proc Am Assoc Cancer Res and ASCO 17:208, 1976.
4. GRALLA RJ, CURRIE VE, WITTES RE, ET AL. Phase II evaluation of pyrazofurin in patients with carcinoma of the lung. Cancer Treat Rep 62:451–452, 1978.
5. GRALLA RJ, SORDILLO PP, and MAGILL GB. Phase II evaluation of pyrazofurin in patients with metastatic sarcoma. Cancer Treat Rep 62:1573–1574, 1978.
6. BUDMAN D, CURRIE V, and WITTES R. Phase II trial of pyrazofurin in malignant melanoma. Cancer Treat Rep 61:1733–1734, 1977.
7. CREAGAN ET, RUBIN J, MOERTEL CG, ET AL. Phase II study of pyrazofurin in advanced colorectal carcinoma. Cancer Treat Rep 61:491–493, 1977.
8. GEHAN EA. The determination of the number of patients required in a preliminary and a follow-up trial of a new chemotherapeutic agent. J Chronic Dis 13:346–353, 1961.

Phase I–II Study of Hexamethylmelamine Plus Dibromodulcitol in Refractory Carcinoma of the Breast [1,2]

Brief Reports and Preliminary Communications

Albert L. Van Amburg III,[3] Cary A. Presant,[3,*] Enrique Vélez-García,[4] and Carol Klahr[3]

The treatment of drug-resistant carcinoma of the breast has been generally disappointing. Commonly encountered problems include poor performance status, lack of effective agents, and paucity of venipuncture sites. Hexamethylmelamine has been shown to have an overall response rate of 25% in phase II trials (1), although response rates have varied from 0 to 32% in individual studies (2–4). In those studies, hexamethylmelamine was given at a dose of approximately 300 mg/m^2 for an indefinite period. Toxicity included bone marrow suppression and neurologic and gastrointestinal side effects.

Dibromodulcitol is an alkylating agent which had a reported response rate of 39% in a large phase II study (5) when given at a dose of 3.5 mg/kg/day for 42 days. Furthermore, responses were seen in patients refractory to other alkylating agents (6). The usual dose-limiting toxicity was myelosuppression with very little nausea and vomiting.

Based on the above results, a phase I–II study was undertaken to determine the toxicity and efficacy of these two experimental antitumor agents, hexamethylmelamine and dibromodulcitol, which can be given orally.

METHODS AND MATERIALS

Patients with metastatic carcinoma of the breast were eligible if resistance to adriamycin, cyclophosphamide, and methotrexate had been demonstrated. All patients had a Karnofsky performance status > 40 and had recovered from previous myelosuppressive therapy. Voluntary informed consent had been given prior to treatment.

The initial dose of both agents was 100 mg/m^2 orally each given daily for 10 days and repeated every 3 weeks. The dose was increased to 125 mg/m^2 of each drug after two courses if there was no significant toxicity. Therapy was delayed until the wbc count was > 4000/mm^3 and the platelet count was $> 100{,}000$/mm^3. A partial response was defined as a $> 50\%$ decrease in the sum of the products of perpendicular tumor diameters.

RESULTS AND DISCUSSION

Sixteen patients with metastatic carcinoma of the breast were entered in the study. There were 15 females and one male. The average age was 62 years with a range of 32–72. Eleven of the 16 patients were ≥ 50 years of age. Fifteen of the 16 patients were evaluable for antitumor response; the other patient was lost to followup and was presumed dead.

Prior chemotherapy had been given to all patients. Three patients had become resistant to one prior combination regimen, six had received two combinations, and six had received three combinations. Fourteen had received cyclophosphamide, methotrexate, and 5-fluorouracil, and 13 had become resistant to chemotherapy combinations containing adriamycin. Four additional patients had also failed to respond to single-drug regimens prior to this study. None of the 15 evaluable patients had a partial response, but two patients had stable disease for 2 and 5+ months (table 1). The median survival time of those patients with progressive disease was 2 months, while the two patients with stable disease remain alive at 5 and 6 months.

There were 32 courses of hexamethylmelamine and dibromodulcitol given to these 16 patients, and 28 were evaluable for toxicity. Twenty-eight courses

[1]Received Sept 5, 1978; revised Nov 17, 1978; accepted Nov 27, 1978.

[2]Supported in part by the Multimodality Oncology Project of The Jewish Hospital of St. Louis, and Public Health Service grants RR-00036 (Division of Research Resources) and CA-13053 and CA-12223 (National Cancer Institute) from the National Institutes of Health, Department of Health, Education, and Welfare. This study was protocol 77P-BR0201 of the Southeastern Cancer Study Group.

[3]Division of Hematology-Oncology, The Jewish Hospital of St. Louis, Washington University School of Medicine, Mo.

[4]Division of Hematology and Medical Oncology, University Hospital, University of Puerto Rico, San Juan.

*_Reprint requests to:_ Cary A. Presant, MD, Division of Hematology-Oncology, The Jewish Hospital of St. Louis, 216 S Kingshighway, St. Louis, Mo 63110.

TABLE 1.—Antitumor response

Response	No. of patients	Duration of response (mos)	Median survival time (mos)
Partial	0	—	—
Stable disease	2	2, 5+	5, 6
Progression	10	—	2.0

were given at a dose of 100 mg/m^2 and four were given at a dose of 125 mg/m^2 of each drug. The dose-limiting toxicity was gastrointestinal. Most patients experienced nausea and eight had moderate to severe vomiting or diarrhea. Patients at the higher dose seemed to have more severe vomiting which responded less well to antiemetic therapy.

Myelosuppression was not commonly seen. Only one patient had a nadir wbc count < 500/mm^3. Four patients had thrombocytopenia (55,000–90,000 cells/mm^3) and three of these received a dose of 125 mg/m^2 of each drug. There was no life-threatening bleeding or infection. Myelosuppression was seen in the first course of therapy in one patient and in subsequent courses in three patients. One patient had visual hallucinations during the fourth course of chemotherapy which cleared after the drugs were discontinued.

CONCLUSIONS

The combination of hexamethylmelamine and dibromodulcitol was not effective in producing tumor regression or palliation in these previously treated patients, despite doses that produced moderate gastrointestinal toxicity. With no partial responses in 15 evaluable patients, it may be concluded that there is a < 5% probability that the response rate would be ≥ 22% (if additional patients were studied).

REFERENCES

1. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine. Cancer 38:27–35, 1976.
2. WILSON WL, BISEL HF, COLE D, ET AL. Prolonged low-dosage administration of hexamethylmelamine (NSC 13875). Cancer 25:568–570, 1970.
3. WAMPLER GL, MELLETTE SJ, KUPERMINC M, ET AL. Hexamethylmelamine (NSC 13875) in the treatment of advanced cancer. Cancer Chemother Rep 56:505–514, 1972.
4. DENEFRIO JM, and VOGEL CL. Phase II study of hexamethylmelamine in women with advanced breast cancer refractory to standard cytotoxic therapy. Cancer Treat Rep 62:173–175, 1978.
5. ANDREWS NC, WEISS AJ, WILSON W, ET AL. Phase II study of dibromodulcitol (NSC-104800). Cancer Chemother Rep 58:653–660, 1974.
6. ANDREWS NC, WEISS AJ, ANSFIELD FJ, ET AL. Phase I study of dibromodulcitol (NSC-104800). Cancer Chemother Rep 55:61–65, 1971.

Vincristine Effect on Methotrexate Cerebrospinal Fluid Concentration [1]

Brief Reports and Preliminary Communications

F. Tejada* and C. G. Zubrod [2]

SUMMARY

A 67-year-old man with malignant lymphoma, nodular, poorly differentiated lymphocytic type, developed meningeal lymphomatosis. Repeated high-dose methotrexate-citrovorum factor treatment (1 g/m² in 24 hours) rendered the patient free of disease. The administration of vincristine at 23 hours consistently increased the concentration of methotrexate in the cerebrospinal fluid 2.5-fold above baseline values.

[Cancer Treat Rep 63:143–145, 1979]

Previous information has indicated that high-dose systemic methotrexate-citrovorum factor (MTX–CF) therapy is effective in the prophylaxis and treatment of central nervous system leukemia (1) and lymphoma (2). Enhancement of MTX intracellular concentration in human neoplastic cells by vincristine has been described (3). We report a case in which enhancement of MTX cerebrospinal fluid (CSF) concentration was seen by the administration of vincristine during high-dose systemic MTX–CF treatment.

CASE REPORT

This 67-year-old white man was well until February 1975 when he discovered a mass in the left side of his neck. An evaluation, including a bone marrow and liver biopsy, rendered the diagnosis of stage IIIA malignant lymphoma, nodular, poorly differentiated lymphocytic type. The patient initially received treatment with monthly cycles of cyclophosphamide, vincristine, and prednisone (CVP regimen) and did well until April 1976, when he complained of headache and light headedness with subsequent development of diplopia and dysequilibrium. He was found to have left III, IV, and VI cranial nerve paresis. CSF cytology showed lymphoid cells consistent with meningeal lymphomatosis. Brain scan, arteriogram, and computerized axial tomographic scan were negative. The patient was treated with 3000 rads of radiation, over a 10-day period, to the whole brain and CVP was continued with disappearance of neurologic signs. In July 1976, the patient complained of numbness in both feet and hands and dysequilibrium which progressed to pain and weakness in the right leg with radiation down to the toe. An examination showed right leg paresis and in September 1976 a myelogram showed marked asymmetry of the nerve roots with filling defects on the right side of the mid-sacral level. The patient was treated with 3000 rads of radiation over a 10-day period (September 21 to October 1, 1976) to a posterior sacral field and his chemotherapy was changed to 100 mg of procarbazine orally daily and 7.5 mg of bleomycin im twice a week. On October 26, 1976, the patient was referred to our Center without improvement and with the appearance of left leg pain and weakness. An examination demonstrated a chronically ill man with a weight of 142 lbs, a palpable spleen tip, and a liver span of 7 cm, but no adenopathy. A neurologic examination showed an unsteady gait because of right leg weakness, and normal cranial nerve and cerebellar function. There was decreased pin prick, touch, and positional sense in fingers and below the knees. Motor strength was decreased in both legs (more on the right). Admission laboratory tests were: hematocrit, 39.7%; hemoglobin, 14.2 g/100 ml; wbc count, 3500/mm³ with normal differential; platelet count, 171,000/mm³; BUN, 18 mg/100 ml; creatinine, 1.5 mg/100 ml; creatinine clearance, 80 ml/minute; normal electrolytes; and normal liver function tests. On admission, treatment with procarbazine and bleomycin was discontinued and an evaluation showed normal serum and urine protein electrophoresis and immunoelectrophoresis. Nerve conduction and electromyogram studies indicated polyneuropathy consistent with vincristine effects. Bilateral posterior iliac

[1]Received Sept 8, 1978; revised Nov 15, 1978; accepted Nov 27, 1978.

[2]Comprehensive Cancer Center for the State of Florida and Department of Oncology, University of Miami School of Medicine.

**Reprint requests to:* Francisco Tejada, MD, Comprehensive Cancer Center for the State of Florida, Roof Garden Centre House, 1400 NW 10th Ave, Miami, Fla 33136.

crest marrow biopsies were hypocellular with no evidence of malignant lymphoma. A bipedal lymphangiogram was normal, with bone and gallium scans showing abnormal uptake in the body of T_2 and L_4, which on the roentgenogram was consistent with osteoporotic compression. A CSF evaluation on October 29, 1976, showed a clear colorless fluid with a protein of 115 mg/100 ml and 4 wbcs/mm^3 which on cytocentrifugation were seen as clefted immature lymphocytes similar to those present in the initial lymph node biopsy. A repeat myelogram was obtained on November 9, 1976, which did not show evidence of extradural mass in the sacral, lumbar, thoracic, or cervical regions. CSF protein was 112 mg/100 ml with positive cytology consistent with lymphoma. With no change in the patient's symptoms an Ommaya reservoir was introduced in the right lateral ventricle on November 16, 1976. On November 22, 1976, 20 mg of MTX was given in the ventricle through the Ommaya reservoir after which the patient complained of continuous nausea and vomiting for 4 days with no further neurologic changes. On November 26, 1976, CSF sampling showed a clear colorless fluid with a protein of 14 mg/100 ml and 1 wbc/mm^3 which was consistent with lymphoma. Serum and CSF MTX concentrations measured by the competitive binding of labeled and unlabeled MTX to the enzyme dihydrofolate reductase (New England Enzyme Center, Boston, Mass) were 0 and 1.9×10^{-8} M, respectively. Because of severe vomiting, the patient was given 1.0 g/m^2 of MTX iv by continuous infusion in 24 hours (priming dose one third of the total calculated dose) followed by CF rescue at 24 hours. The patient tolerated this therapy well with improvement of his right leg weakness and was discharged home on a walker to continue therapy every 2–3 weeks. The highest MTX concentration levels measured at 24 hours during two of these courses of treatment were 1.5×10^{-4} M and 5.5×10^{-7} M for serum and CSF, respectively. In three of the subsequent courses of high-dose MTX–CF treatment the patient received 1.4 mg/m^2 of vincristine by iv push at 23 hours during the MTX infusion. CF rescue was delayed to 30 hours, serum and CSF samples were obtained at 0, 6, 23, 24, 26, 28, and 30 hours, and MTX concentration levels were measured as previously indicated.

The mean serum and CSF values of MTX during these three courses as shown in figure 1 indicate a 2.5-fold increase in the CSF MTX concentration following the vincristine administration. This effect is seen up to 3 hours after the dose of vincristine and for 2 hours after the discontinuation of the MTX infusion. The patient continues receiving MTX–CF therapy and remains free of disease with minimal peripheral neuropathy as the remaining neurologic deficit.

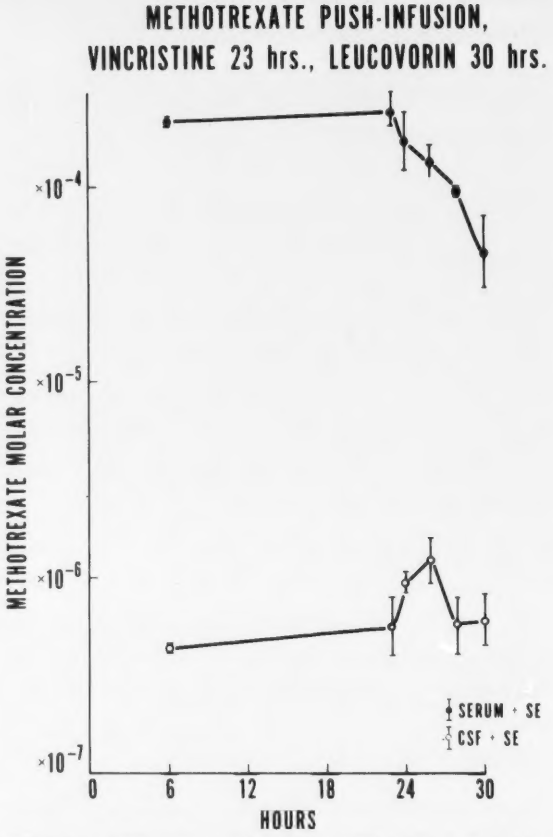


FIGURE 1.—MTX concentration during MTX infusion (1 g/m^2 iv, Hours 0–24) and vincristine administration (1.4 mg/m^2 iv at 23 hrs). Curve indicates the mean ± SE of 3 consecutive courses of treatment.

COMMENT

The accumulation of free intracellular MTX in mammalian cells is restricted, even at MTX concentrations comparable to those used in high-dose MTX–CF protocols (4). Vincristine-induced augmentation of MTX uptake in Ehrlich ascites (4,5) and in human neoplastic (3) cells could be a potentially valuable principle for the combined use of vincristine and MTX if this relationship is valid in the in vivo situation. MTX is a weak organic acid and some organic acids and bases are actively pumped out of the CSF to the blood probably through the metabolic pump in the choroid plexus (6). Vincristine is considered to inhibit similar metabolic pumps (3,5)

in mammalian cells. This case report presents information which seems to indicate that vincristine, at usual therapeutic doses, is able to enhance transmembrane concentrations of MTX in man even at serum concentrations of 10^{-4} M. Furthermore, the duration of the vincristine effect lasts for a few hours, consistent with the rapid α and β phases of vincristine clearance from the blood (7). The exploration of other dose-schedule pharmacokinetic interactions between MTX and the vinca alkaloids may be of importance in the clinical setting, at least as related to increasing the concentration of MTX in the CSF.

REFERENCES

1. FREEMAN AI, WANG JJ, and SINKS LF. High dose MTX (HDM) in acute lymphocytic leukemia (ALL). Proc Am Assoc Cancer Res and ASCO 16:232, 1975.
2. ZUCKERMAN KS, SKARIN AT, PITMAN SW, ET AL. High dose methotrexate with citrovorum factor (MTX/CF) in the treatment of advanced non-Hodgkin's lymphoma (NHL), including CNS involvement. Proc Am Soc Hematol 19:73, 1976.
3. BENDER RA. Membrane transport of methotrexate (NSC–740) in human neoplastic cells. Cancer Chemother Rep Part 2, vol 6(1):73–82, 1975.
4. GOLDMAN ID, GUPTA V, WHITE JC, ET AL. Exchangeable intracellular methotrexate levels in the presence and absence of vincristine at extracellular drug concentrations relevant to those achieved in high-dose methotrexate-folinic acid "rescue" protocols. Cancer Res 36:276–279, 1976.
5. FYFE MJ, and GOLDMAN ID. Characteristics of the vincristine-induced augmentation of methotrexate uptake in Ehrlich ascites tumor cells. J Biol Chem 248:5067–5073, 1973.
6. RALL DP. Experimental studies of the blood-brain barrier. Cancer Res 25:1572–1577, 1965.
7. OWELLEN RJ, ROOT MA, and HAINS FO. Pharmacokinetics of vindesine and vincristine in humans. Cancer Res 37:2603–2607, 1977.

Phase II Study of High-Dose Methotrexate in Patients With Advanced Malignant Melanoma [1]

Brief Reports and Preliminary Communications

Richard I. Fisher,* Bruce A. Chabner, and Charles E. Myers [2]

The median survival time of all patients with disseminated malignant melanoma is only 4 months (1). DTIC and methyl-CCNU (meCCNU) are the chemotherapeutic agents that produce the highest response rate in advanced melanoma; yet only 20% of all patients treated with either of these agents will have an objective response (2). Unfortunately, patients who develop progressive disease while receiving either of these agents usually do not respond when treated with the other drug or any other agent (3). Therefore, the identification of new chemotherapeutic agents that possess significant antimelanoma activity and are not cross-resistant with established agents appears essential before effective combination chemotherapy can be developed for malignant melanoma.

Standard doses of methotrexate have demonstrated little activity against malignant melanoma. Review of the literature identified only two of 25 patients (8%) with responses to conventional-dose methotrexate (4). In 1976, however, Eilber and Isacoff (5) reported that five of 22 patients with disseminated malignant melanoma treated with high-dose methotrexate had complete disappearance of tumor and three additional patients had partial responses. Because of our interest in discovering agents not cross-resistant with existing drugs, we initiated a phase II study of high-dose methotrexate which included patients previously treated with DTIC or meCCNU.

PATIENTS AND METHODS

The phase II study of high-dose methotrexate was initiated in February 1976. All patients had clinically evaluable disease that could not be surgically resected. Normal renal function, defined as a BUN of < 24 mg/100 ml and a creatinine of < 1.4 mg/100 ml, was required.

All patients received 3000 ml/m^2/day of iv fluids beginning 6 hours prior to the methotrexate infusion and continuing until the end of citrovorum factor rescue. Sufficient sodium bicarbonate was added to the iv fluids to maintain urine pH at > 7.0. High-dose methotrexate was given as a 6-hour infusion. Therapy was usually initiated with a dose of 50 mg/kg of methotrexate; in the absence of significant toxicity, doses were increased to 50 mg/kg with each succeeding course to a maximum of 250 mg/kg. In later phases of this study a more rapid escalation of methotrexate dosage was utilized. Two hours following the infusion, citrovorum factor (15 mg/m^2 iv) was given every 6 hours for eight doses. The plasma level of methotrexate was determined at 48 hours as previously reported (6). If the 48-hour methotrexate level was $> 5 \times 10^{-8}$ M, additional citrovorum factor was given. Therapy was given every 21 days until progressive disease, defined as a 50% increase in followable disease, was documented.

The characteristics of the 23 patients with advanced melanoma who were treated with high-dose methotrexate are shown in table 1. There were 14 males and nine females. The median age was 43 years with a range of 17–63. Six patients had disease limited to soft tissue or lymph nodes while 17 patients had visceral metastases. Twenty of the 23 patients had received prior chemotherapy with DTIC or meCCNU. The median Karnofsky score was 80 with a range of 60–100.

TABLE 1.—Patient characteristics

Total No. of patients	23
Sex	
Males	14
Females	9
Median age in yrs (range)	43 (17–63)
Sites of disease	
Soft tissue and lymph nodes only	6
Visceral metastases	17
Prior therapy with DTIC or meCCNU	20
Median Karnofsky score (range)	80 (60–100)

[1]Received Sept 26, 1978; accepted Nov 28, 1978.

[2]Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

Reprint requests to: Richard I. Fisher, MD, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, Md 20014.

RESULTS AND DISCUSSION

No objective responses were seen in 23 patients with advanced malignant melanoma who were treated with high-dose methotrexate. Therapy was discontinued in all patients because of progressive disease. The highest doses of methotrexate received by the patients were as follows: 50 mg/kg (two patients); 100 mg/kg (three patients); 150 mg/kg (three patients); 200 mg/kg (11 patients); and 250 mg/kg (four patients).

Toxicity consisted of the following: minimal nausea and vomiting in three patients, mucositis in five patients, wbc count $< 2000/mm^3$ and platelet count $< 50,000/mm^3$ in four patients, abnormal liver function tests which returned to normal before the next cycle in four patients, and serum creatinine > 2 mg/100 ml in two patients. There were no deaths attributed to drug toxicity. Only ten of the 23 patients had no evidence of toxicity during their entire course of methotrexate.

The results of this study differ significantly from those in the report of Eilber and Isacoff (5). They reported an objective response rate of 36% (eight responses among 22 patients). However, their patients had limited residual tumor and had not received prior chemotherapy. Vincristine (1 mg/m^2) was given prior to each methotrexate infusion in the Eilber and Isacoff study but not in the present trial. The schedule and dose of methotrexate administered appear similar in the two trials. Therefore, on the basis of the current study we cannot rule out the possibility that the responses previously reported might be due to the combination of vincristine and methotrexate. However, the differences in the extent of metastatic tumor and prior chemotherapy appear more likely to explain the discrepancies between the two studies.

The results of this phase II study demonstrate that high-dose methotrexate is not effective in the treatment of patients with advanced melanoma who have failed trials of DTIC or meCCNU. This fact, combined with the significant cost and toxicity of the regimen, makes it unlikely that high-dose methotrexate will have a significant role in the treatment of metastatic melanoma.

REFERENCES

1. EINHORN LH, BURGESS MA, VALLEJOS C, ET AL. Prognostic correlations and response to treatment in advanced metastatic malignant melanoma. Cancer Res 34:1995–2004, 1974.
2. YOUNG RC, CANELLOS GP, CHABNER BA, ET AL. Treatment of malignant melanoma with methyl CCNU. Clin Pharmacol Ther 15:617–622, 1974.
3. AHMANN DL, HAHN RG, and BISEL HF. Evaluation of 1-(2-chloroethyl-3-4-methylcyclohexyl)-1-nitrosourea (methyl-CCNU, NSC 95441) versus combined imidazole carboxamide (NSC 45388) and vincristine (NSC 67574) in palliation of disseminated malignant melanoma. Cancer 33:615–618, 1974.
4. COMIS RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
5. EILBER FR, and ISACOFF W. High dose methotrexate therapy for disseminated malignant melanoma. Proc Am Assoc Cancer Res and ASCO 17:262, 1976.
6. MYERS CE, LIPPMAN ME, ELIOT HM, ET AL. Competitive protein binding assay for methotrexate. Proc Natl Acad Sci USA 72:3683–3686, 1975.

LETTERS

Phase II Study of Nafoxidine in the Therapy for Advanced Renal Carcinoma[1]

Nafoxidine is a nonsteroidal competitive inhibitor of estrogen binding to its receptor in the target tissues. It has been demonstrated to have antitumor activity in dimethylbenzanthracene-induced rat mammary carcinoma (1) and recently in human breast cancer (2). In 1967 Bloom et al (3) showed that nafoxidine induced objective remissions in an estrogen-induced renal cell carcinoma in the Golden hamster. In a randomized study,[2] nafoxidine was shown by the Eastern Cooperative Oncology Group to be superior to oral medroxyprogesterone acetate (Provera) and hydroxyurea in the treatment of progressive human metastatic renal cell carcinoma. Nafoxidine produced a response rate of 15%, compared to the 5% response rate each for medroxyprogesterone acetate and hydroxyurea. However, the conclusion of the group was that there was no significant difference in the three treatments with regard to tumor response or survival since the trial had a small number of patients (20 patients) in each treatment arm and only gross differences could have been detected. We are reporting our experience in a phase II therapeutic trial with nafoxidine in 21 patients with inoperable or metastatic adenocarcinoma of the kidney.

MATERIALS AND METHODS

Twenty-one patients with measurable, unresectable, or metastatic renal cell carcinoma received nafoxidine. Eighteen patients had the diagnosis established histologically by nephrectomized specimens during the operations or by kidney biopsy. The three remaining patients had presumptive evidence of renal cell carcinoma with characteristic hypervascular renal masses demonstrated by arteriography. Nafoxidine was administered as a daily dose of 180 mg divided into three equal doses taken with meals. Patients were advised to avoid exposure to the sun to prevent skin dryness. After February 1, 1977, vitamin A (25,000 units/day) was given to three patients by their physicians to prevent ichthyosis. Toxicity and response were evaluated in most patients at 2–4-week intervals. Metastatic bone survey, chest roentgenogram, and liver and brain radioisotopic scans were obtained as clinically indicated to evaluate response. Therapeutic responses were evaluated in the following manner: complete response was defined as total regression of all measurable lesions, partial response (PR) as a measurable decrease of $> 50\%$, and improvement as any response of $< 50\%$. The Karnofsky performance status was used to evaluate subjective response.

Patient Characteristics

Preoperative radiotherapy to the renal bed was given to one of the patients. Four other patients had postoperative radiotherapy to the involved renal bed. Ten patients were treated with radiotherapy to their metastatic sites. The rest of the patients did not receive any radiation therapy. There were four patients with clear cell carcinoma, two patients with spindle variant carcinoma, and two patients with mixed clear cell and granular carcinoma; the remaining patients had adenocarcinoma. Six patients had received medroxyprogesterone acetate alone and three had received chemotherapy alone with no response prior to the trial of nafoxidine. Three patients had failed to respond to medroxyprogesterone acetate in addition to chemotherapy prior to nafoxidine. There were no previous hormonal responses in any of the patients who had received medroxyprogesterone acetate. Nine patients received no previous hormonal therapy or chemotherapy. There were 12 male and nine female patients in our study. The median age was 54 years (range, 34–75 years). The sites of evaluable lesions included lung (15 patients), liver (four), bone (nine), brain (two), lymph nodes or subcutaneous mass (two), and abdominal mass (two). Two patients had been treated with mithramycin for hypercalcemia prior to receiving nafoxidine.

Responses

One patient died 3 weeks after therapy was started and although she was not considered to

[1]Supported in part by Public Health Service grant CA–17952 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare. The views expressed are those of the authors and should not be considered in any way to represent the views of the National Cancer Institute.

[2]Eastern Cooperative Oncology Group Protocol No. 0269. Oral Provera, hydroxyurea and U–11, 100A in renal cell carcinoma. Group minutes, Sept 1973.

TABLE 1.—Toxicity from nafoxidine therapy

Toxic effect	No. of patients
Mild leukopenia (wbc count, 3000–4000/mm³)	4
Moderate thrombocytopenia (platelet count, 50,000–74,999/mm³)	1
Ichthyosis	3
Gastrointestinal disturbance (nausea, diarrhea, and vomiting)	3
Hypercalcemia	2

have had an adequate trial, she did not show any benefit from nafoxidine. Of the 20 patients who were evaluable, one patient had a PR for 6 months with a > 50% decrease in the size of her pulmonary nodules but afterwards the disease progressed. One patient showed improvement in her lung metastases for a period of 3 months. Both patients also had subjectively improved performance status. These two patients had received no prior hormonal therapy or chemotherapy. Six patients showed no change in their disease either objectively or subjectively and the remaining patients showed increasing disease. The response rate was 5%.

Toxicity

The most common toxic effect (table 1) was mild leukopenia which developed in four patients who were receiving nafoxidine. One of these patients also developed moderate thrombocytopenia but was receiving radiotherapy to the mediastinum at the same time. Two patients developed hypercalcemia while receiving nafoxidine but both patients had been treated previously for hypercalcemia prior to nafoxidine therapy.

DISCUSSION

Nafoxidine does not appear to be a useful agent in the treatment of advanced metastatic renal cell carcinoma. Our series showed only a 5% response rate with one PR in 20 evaluable patients. In the Eastern Cooperative Oncology Group study in which nafoxidine was administered at a dose of 4 mg/kg/day orally as a single dose, the response rate was 15% with one PR and two complete responses in 20 patients.

The drug was reasonably well-tolerated in our series. Hematologic toxic effects included mild leukopenia in four patients and moderate thrombocytopenia in one patient. Gastrointestinal toxic effects and ichthyosis were mild and generally tolerable.

The efficacy of hormonal therapy for metastatic or unresectable renal cell carcinoma has been recently challenged (4). Nafoxidine, an antiestrogen agent, in our experience has proven to be of little therapeutic value in the management of this cancer.

REFERENCES

1. GALLEZ G, HEUSON J-C, and WAELBROECK C. Growth-stimulating effect of nafoxidine on rat mammary tumor after ovariectomy. Eur J Cancer 9:699–700, 1973.
2. TERENIUS L. Anti-oestrogen and breast cancer. Eur J Cancer 7:57–64, 1971.
3. BLOOM HJG, ROE FJC, and MITCHLEY BCV. Sex hormones and renal neoplasia. Inhibition of tumor of hamster kidney by an estrogen antagonist, an agent of possible therapeutic value in man. Cancer 20:2118–2124, 1967.
4. PROUT GR, JR. The kidney and ureter. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Lea and Febiger, 1974, pp 1655–1669.

Lynn G. Feun
Anibal Drelichman
Amnuay Singhakowinta
Vainutis K. Vaitkevicius

Department of Oncology
Wayne State University School
of Medicine
and Harper-Grace Hospitals
540 E Canfield
Detroit, Mich 48201

Noboru Oishi

Clinical Sciences Unit
Cancer Center of Hawaii
Honolulu, Hawaii

October 24, 1978

Systemic Allergic Reaction to Adriamycin[1]

Since the commonly used antineoplastic agent adriamycin is an anthracycline antibiotic product of *Streptomyces peucetius* (1), allergic reactions might be expected. Reports of hypersensitivity responses, however, have been extremely limited. Isolated reports describe primarily patchy erythema and/or urticaria along the course of veins used for injection. Etcubanas and Wilbur (2) mentioned a patient who developed anaphylaxis associated with the above response but did not discuss it further. Single occurrences of conjunctivitis and urticaria were listed by van Dyk et al (3) as "toxic" reactions to adriamycin without further elaboration. An isolated case of contact dermatitis was observed in a technician who had been working with anthracycline

[1]The opinions or assertions contained in this paper are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

antibiotics (4). The following case report is the first detailed clinical description of a systemic allergic reaction to adriamycin.

CASE REPORT

A 49-year-old female presented in January 1977 with stage III undifferentiated large cell bronchogenic carcinoma. Initial treatment consisted of split-course radiotherapy to the primary lesion and mediastinum. The patient began to receive combination chemotherapy with methotrexate, adriamycin, cyclophosphamide, and CCNU every 3 weeks as reported by Chahinian et al (5). She tolerated the first three cycles of chemotherapy well without any evidence of local or systemic reaction. No local reaction to the adriamycin injection during the fourth cycle was observed. However, 3 days later, the patient noted the onset of burning and swelling of her eyes. The following day she developed a diffuse urticarial skin eruption. All signs and symptoms subsided spontaneously over the next 24 hours. One hour after her fifth course of chemotherapy she developed transient pruritis of the head, neck, and upper extremities. This was accompanied by marked angioedema of the eyelids and tongue which was associated with difficulty in swallowing. The swelling gradually subsided over the next 2 weeks. A similar reaction occurred after her sixth treatment.

Scratch and intradermal skin tests were negative to routine seasonal and perennial allergens. A positive control test with a histamine base, 0.1 mg/ml, produced an 8-mm wheal with 20 mm of light erythema. Scratch and intradermal skin tests with methotrexate, cyclophosphamide, and CCNU were also negative. Skin testing was not performed with adriamycin because this agent is known to cause severe local tissue necrosis when injected into the skin (1).

After informed consent was obtained, the patient was hospitalized for diagnostic challenge with each of the individual chemotherapeutic agents administered in fractional doses. Full preparations were made for the management of any resultant anaphylactic reaction. The patient tolerated the separate diagnostic challenges with cyclophosphamide, then methotrexate, followed by CCNU on separate successive days without any difficulty. On the fourth day, 2 minutes after iv administration of 14 mg of adriamycin, the patient began to complain of facial pruritis, became agitated, and developed marked respiratory distress. Auscultation of the chest revealed diffuse wheezing. No urticaria or reaction along the route of injection was noted. The pulse rate increased from 84 to 126 beats/minute. The blood pressure decreased from a baseline of 130/80 to 110/50 mm of mercury. Aqueous epinephrine and hydrocortisone acetate were administered iv. Immediate improvement was noted. Within 20 minutes, all symptoms had subsided and vital signs had returned to baseline values.

Five subsequent courses of methotrexate, cyclophosphamide, and CCNU without adriamycin were administered at 3-week intervals without any evidence of allergic reactions.

COMMENT

This report documents the second published case of a systemic allergic reaction to adriamycin. We discussed our experience with this patient with other oncologists and clinical immunologists; some mentioned similar reactions to combination chemotherapeutic regimens which included adriamycin. Potentially life-threatening allergic reactions to adriamycin, thus, may be more common than have been previously recognized. Physicians prescribing the anthracycline antibiotics such as adriamycin or daunorubicin should be aware of this possible risk.

REFERENCES

1. BLUM RH, and CARTER SK. Adriamycin: a new anticancer drug with significant clinical activity. Ann Intern Med 80:249–259, 1974.
2. ETCUBANAS E, and WILBUR JR. Letter: Uncommon side effects of adriamycin (NSC–123127). Cancer Chemother Rep 58:757–758, 1974.
3. VAN DYK JJ, VAN DER MERWE AM, FALKSON HC, ET AL. Adriamycin in the treatment of cancer. S Afr Med J 50:61–66, 1976.
4. REICH SD, and BACHUR NR. Letter: Contact dermatitis associated with adriamycin (NSC–123127) and daunorubicin (NSC–82151). Cancer Chemother Rep 59:677–678, 1975.
5. CHAHINIAN AP, ARNOLD DJ, COHEN JM, ET AL. Chemotherapy for bronchogenic carcinoma: methotrexate, doxorubicin, cyclophosphamide and lomustine. JAMA 237:2392–2396, 1977.

Daniel J. Arnold
Chester T. Stafford*

Department of Medicine
Dwight D. Eisenhower Army
Medical Center
Fort Gordon, Ga 30905

October 30, 1978

*To whom reprint requests should be addressed.

Use of Combination Chemotherapy With CCNU, Bleomycin, and Vincristine in the Treatment of Metastatic Melanoma in Patients Resistant to DTIC Therapy

The favorable response rates reported in the chemotherapy for stage III melanoma by Moon (1) employing vincristine (VCR) and BCNU and by De

TABLE 1.—Characteristics of responders and nonresponders to CCNU, VCR, and bleomycin

Patient No.	Age (yrs)	Sex	Sites of metastases	Previous DTIC treatment	Response to DTIC	No. of cycles of CCNU, VCR, and bleomycin	Response and duration	Survival (wks)
				Responders				
1	55	M	Subcutaneous nodules, lymph nodes, and ascites	250 mg/m² × 5 days × 4 mos; nodes and subcutaneous nodules until ascites developed	None	2.5	Partial for 6 wks: > 50% reduction of nodes and subcutaneous nodules and complete resolution of ascites	16
2	70	M	Subcutaneous nodules, lymph nodes, pulmonary, and ascites	250 mg/m² × 5 days × 5 mos until ascites developed	None	2.5	Partial for 6 wks: > 50% reduction in size of subcutaneous nodules and nodes and complete resolution of ascites	16
3	35	M	Intradermal and subcutaneous nodules and pleural effusion	250 mg/m² × 5 days × 4 mos; intradermal and subcutaneous nodules then effusion developed	None	4	Complete resolution of pleural effusion and 90% reduction of dermal and subcutaneous nodules for 14 wks	46
4	25	F	Subcutaneous nodules and lymph nodes	250 mg/m² × 5 days × 4 mos	None	2.5	Partial: > 50% reduction in subcutaneous nodules and nodes for 3 mos	20
				Nonresponders				
5	70	M	Subcutaneous and intradermal nodules, lymph nodes, and pulmonary	250 mg/m² × 5 days × 4 mos	None	1	None	6
6	25	M	Intradermal and subcutaneous nodules, lymph nodes, kidneys, ureters, bladder, and urethra	250 mg/m² × 5 days × 3 mos	None	3	None	28
7	23	M	Lymph nodes, pulmonary, and hepatic	250 mg/m² × 5 days × 3 mos	None	1.5	None	12
8	62	M	Lymph nodes, intradermal and subcutaneous nodules, pulmonary, and carcinomatosa lymphangitis	250 mg/m² × 5 days	Partial response, then disease progressed	2	None	Alive at 12

9	72	M	Bone, pulmonary, and lymph nodes	250 mg/m² × 5 days × 3 mos	None	1	None	7
10	46	F	Lymph nodes, subcutaneous nodules, hepatic, and bone	250 mg/m² × 5 days × 3 mos	None	1	None	9
				Nonresponders with cerebral metastases				
11	70	M	Pulmonary and cerebral	250 mg/m² × 5 days × 4 mos until cerebral metastases developed	None	< 1	None	4
12	68	M	Pulmonary and cerebral	250 mg/m² × 5 days × 3 mos until cerebral metastases developed	None	< 1	None	5
13	55	F	Cerebral	250 mg/m² × 5 days × 3 mos	None	2	None	17

Wasch et al (2) using CCNU, VCR, and bleomycin, together with disappointing results of chemotherapy (approximately 10% response) with DTIC in our own patients, led us to treat patients who had stage III melanoma with CCNU, VCR, and bleomycin. A 40% response rate was obtained. All patients had previously been treated with DTIC.

A total of 13 patients with progressive disease, ten males and three females, have so far been treated and followed up for a minimum of 3 months or until death. Twelve patients were resistant to DTIC and one relapsed while receiving DTIC given in a dose of 250 mg/m² for 5 consecutive days; the course was repeated every 28 days.

CCNU was given at a dose of 130 mg/m² orally every 6 weeks, VCR at a dose of 1.4 mg/m² (maximum dose unit, 2 mg), and bleomycin at a dose of 10 mg/m² (maximum dose unit, 15 mg, and maximum total dose, 300 mg), with the latter two drugs being given iv weekly for up to 20 weeks. VCR was discontinued in the event of neurotoxic effects if they were more severe than paresthesia and loss of deep tendon reflexes. A constant watch was kept for pulmonary toxicity with bleomycin.

Only objective criteria of response were used. Complete response was defined as the disappearance of all manifestations of disease. Partial response (PR) was defined as a regression of 50% of the size of measurable lesions (measured clinically or radiologically) lasting for at least 1 month without progression of disease or the appearance of any new lesions at any site. Table 1 details the previous chemotherapy, sites of metastases, and responses of the patients to the CCNU, VCR, and bleomycin regimen.

There were ten patients, eight males ranging in age from 23 to 70 years (mean, 40 years) and two females aged 27 and 47 years (mean, 37 years), who had cutaneous, subcutaneous, lymph node, or visceral metastases with no detectable central nervous system disease; three patients had clinical evidence of cerebral metastases supported by appropriately positive brain scans. Of the ten patients with non-central nervous system disease, three had ascites or pleural effusions in addition to cutaneous, subcutaneous, and lymph node metastases and one had only subcutaneous and lymph node disease when combination chemotherapy was started.

No patient experienced a complete regression but four of the ten patients with non-central nervous system disease had PRs (table 1) lasting from 6 weeks to 4 months including complete disappearance clinically and radiologically of a pleural effusion together with a 90% regression of subcutaneous and intradermal metastases for 14 weeks in one male patient and complete disappearance of ascites in two patients. The one patient with nonvisceral metastases experienced a PR. Six patients did not respond; this group included two patients each having several metastases to the bone (table 1). The mean survival was 25 weeks (range, 16–46 weeks) for responders and 13 weeks (range, 6–28 weeks) for nonresponders. The three patients with cerebral metastases did not respond and had a mean survival of 8 weeks (range, 4–17 weeks). Two of these patients did not complete one full cycle of combination chemotherapy (table 1).

Four patients experienced neurotoxic effects after two or more cycles and in two they were severe enough to warrant cessation of VCR. Seven patients experienced transient hematologic toxic effects: platelet count $< 100{,}000/mm^3$, fall in hemoglobin of 2 g/100 ml, or leukopenia of < 4000 cells/mm^3. In only one patient was there severe but still transient leukopenia, ie, < 1000 wbcs/mm^3. Gastrointestinal intolerance to CCNU was observed in all patients but, since this drug was given at intervals of 6 weeks, the patients did not find this too incapacitating. Six patients had moderate alopecia and two had severe alopecia. Pulmonary toxicity due to bleomycin was not encountered. Overall, the treatment was well-tolerated by the patients, with neurotoxicity causing the most discomfort.

When the patients with cerebral metastases (who in all series are very unresponsive to chemotherapy) are excluded, these results represent a 40% response rate in a predominantly male (80%) series of patients with metastases at more than one site previously treated with DTIC. There was no evidence of cross-resistance with DTIC. A combination of CCNU and procarbazine (Didolkar et al [3]) in patients resistant to DTIC produced poor results and it was therefore suggested that "second attempt" chemotherapy in advanced melanoma resulted in poor response. Our results correspond well with those of De Wasch et al (2) and are, we feel, sufficiently encouraging to warrant more widespread use of this combination of marginally effective drugs (CCNU, VCR, and bleomycin used as single agents giving response rates in metastatic melanoma of 13%, 12%, and 0, respectively) in the therapy for metastatic melanoma, both as a first-line therapy and in those patients previously treated with DTIC. It would appear that, at least with the combination reported here, second attempt chemotherapy can achieve remissions and is worthy of further investigation in order to evaluate its efficiency in a larger number of patients.

REFERENCES

1. MOON JH. Combination chemotherapy in malignant melanoma. Cancer 26:468–473, 1970.

2. DE WASCH G, BERNHEIM J, MICHEL J, ET AL. Combination chemotherapy with three marginally effective agents, CCNU, vincristine, and bleomycin, in the treatment of stage III melanoma. Cancer Treat Rep 60:1273–1276, 1976.
3. DIDOLKAR MS, BAFFI RR, CATANE R, ET AL. Letter: Use of methyl-CCNU and procarbazine in advanced malignant melanoma resistant to DTIC therapy. Cancer Treat Rep 61:1738–1739, 1977.

J. D. Everall
P. M. Dowd

Skin Department
The Royal Marsden Hospital
Fulham Road
London SW3, England

October 30, 1978

Nonresponsiveness of Brain Tumors to VM-26 Therapy in Children

The activity of the semisynthetic podophyllotoxin derivative, VM-26, is being studied throughout the pediatric tumor spectrum by the Pediatric Division of the Southwest Oncology Group. This agent, which is lipid-soluble, has shown only low-level activity against ic inoculated L1210 leukemia (1). In man, the agent passes the blood-brain barrier poorly and cerebrospinal fluid levels are < 1% of the plasma levels (2). Even so, some activity was noted in earlier studies in patients with brain tumors (3,4). An adequate number of children with brain tumors have now completed the Southwest Oncology Group's drug trial permitting assessment of the efficacy and toxicity of VM-26 in this diagnostic category in children.

VM-26 was given at a dose of 45 mg/m²/day iv × 5 days. A second course was to be given in 28 days. Doses for the second and subsequent courses were adjusted as follows: for wbc count nadir ≥ 5500/mm³, increase dose by 33.3%; for wbc count nadir 3500–5500/mm³, no change in dose; for wbc count nadir 1500–3500/mm³, decrease dose by 33.3%; and

TABLE 1.—Diagnoses, prior therapy, and methods of evaluating patients receiving ≥ 2 courses of VM-26

Patient No.	Diagnosis	Prior therapy*	Time from diagnosis to VM-26	Methods of evaluation†
1	Brain stem glioma	Radiotherapy: 6000 rads to brain and 2400 rads to spine	4½ mos	Electroencephalogram, echoencephalogram, pneumoencephalogram, cerebral angiography, neurologic examinations, and autopsy
2	Medulloblastoma	Radiotherapy: 3500 rads to cerebellum and 2500 rads to spine; CCNU, VCR, and intrathecal MTX	2 mos	Electroencephalogram, brain scan, skull films, cerebrospinal fluid examinations, and neurologic examinations
3	Astrocytoma, grade II	Partial excision of tumor; radiotherapy: 5300 rads to brain; dexamethasone and ICRF-159	2 mos	Neurologic examinations
4	Medulloblastoma	Excision of left cerebellar hemisphere; radiotherapy; VCR, BCNU, and dexamethasone	9½ mos	Ventriculogram, pneumoencephalogram, skull films, cerebrospinal fluid examinations, and neurologic examinations
5	Glioma	Radiotherapy: 4600 rads to brain and 1050 rads to brain; VCR and CYT	7½ mos	Pneumoencephalogram, angiography, CT scans, and neurologic examinations
6	Brain stem glioma	Radiotherapy to brain; adriamycin, DTIC, VCR, and CYT	1⅓ yrs	Arteriogram and neurologic examinations
7	Glioma, hypothalamus	Partial excision; radiotherapy: 4200 rads; CCNU and ICRF-159	3¼ yrs	CT scans and neurologic examinations
8	Medulloblastoma	Excision biopsy; radiotherapy: 5000 rads to brain and 3000 rads to spine; VCR, CYT, and MTX	1 yr	CT scans and neurologic examinations
9	Thalamic tumor	Radiotherapy: 4500 rads to parasellar region	1¹⁰/₁₂ yrs	Pneumoencephalogram, CT scans, and carotid and vertebral arteriograms
10	Brain stem glioma	Radiotherapy to brain stem	3 mos	CT scan and neurologic examinations

*VCR = vincristine; MTX = methotrexate; CYT = cyclophosphamide.
†CT = computerized tomography.

for wbc count nadir < 1500/mm^3, reduce dose by 50%.

Seventeen children with brain tumors were entered in the study. In each of these patients, clinical investigations gave assurance of residual or recurrent tumor in the central nervous system. The age of the patients ranged from 2 to 15 years (median, 6 years). The number of children in each diagnostic category was as follows: medulloblastoma, eight; astrocytoma, three; brain stem glioma, three; ependymoma, one; glioma of the hypothalamus, one; and thalamic tumor, one. The time from diagnosis to VM-26 therapy ranged from 3 to 39 months (median, 15 months). All children had received prior radiotherapy and three had received no prior chemotherapy. The remaining 14 children had received both prior chemotherapy and radiotherapy. Vincristine had been included among the agents given to 12 of the children.

Ten patients received two or more courses of VM-26 and were considered evaluable for drug effect. For each of these patients, diagnosis, prior therapy, time from diagnosis to VM-26 therapy, and methods of evaluation are shown in table 1. Four patients completed only one course of VM-26. One patient was lost to followup. In two children early death prevented completion of a course of treatment. The response to VM-26 therapy was evaluated by careful neurologic examinations and selected clinical studies thought to be most appropriate for the clinical status of the particular patient. A specific battery of tests was not required on a fixed schedule.

There was no significant clinical improvement in the ten evaluable patients following two or more courses of VM-26. One patient had slight improvement in cerebellar function. One patient received multiple courses of treatment and developed leukopenia with a wbc count nadir of 300/mm^3 on Day 7. Recovery followed in 5 days. This patient had received radiotherapy to the cranium and to the pelvis. Treatment with vincristine, procarbazine, and methotrexate had been completed 4 weeks prior to VM-26 therapy.

A zero response rate in the ten patients would be expected to occur < 11% of the time when the true complete remission rate was ≥ 20%. From the data presented, it is concluded that VM-26, administered as described, has a very high probability of being ineffective in treating brain tumors in children.

REFERENCES

1. VENDITTI JM. Treatment schedule dependency of experimentally active antileukemic (L1210) drugs. Cancer Chemother Rep Part 3, vol 2(1):35–59, 1971.
2. CREAVEN PJ, and ALLEN LM. PTG, a new antineoplastic epipodophyllotoxin. Clin Pharmacol Ther 18:227–233, 1975.
3. MUGGIA FM, SELAWRY OS, and HANSEN HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.
4. SKLANSKY BD, MANN-KAPLAN RS, REYNOLDS AF, JR, ET AL. 4′-Demethyl-epipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.

Margaret P. Sullivan
Jan van Eys
Jay Herson
Kenneth A. Starling
Abdel Ragab
Charles Sexhauer

Pediatric Division
Southwest Oncology Group*
Operations Office
3500 Rainbow Blvd, Suite 100
Kansas City, Kan 66103

November 3, 1978

*Reprint requests should be addressed to the Southwest Oncology Group.

Combination 6-Mercaptopurine and 6-Methylmercaptopurine Riboside in the Treatment of Adult Acute Leukemia: A Southwest Oncology Group Study[1]

This study was developed in 1967 to determine whether the combination of 6-mercaptopurine (6-MP) and 6-methylmercaptopurine riboside (6-MMPR) would be superior to 6-MP alone in the treatment of adult acute leukemia. The response of acute myelogenous leukemia (AML) to 6-MP is low, with only 9%–11% achieving complete remission (CR). Both 6-MP and 6-MMPR are effective in L1210 leukemia and have shown synergism when used in combination (1). Resistance to 6-MP can develop in L1210 leukemia, which is then resistant to all analogs of 6-MP (2). 6-MMPR is also active in L1210 leukemia resistant to 6-MP; unlike 6-MP it does not require inosinic acid pyrophosphorylase for conversion to the active nucleotide (2). Resistance to 6-MP in L1210 leukemia is associated with a loss of inosinic acid phosphorylase whereas 6-MP in human leukemia has not been associated with deletion of this enzyme (3). The mechanism of resistance may be different in experimental and human systems. However, since the biochemical basis for resistance to 6-MP in human leukemia is unknown and synergism with the combination of 6-MP and 6-MMPR is

[1]Supported by Public Health Service grants CA-04919, CA-10376, CA-04920, CA-03389, CA-16943, and CA-12014 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

reported in L1210 leukemia, a study of this combination seemed indicated.

The toxic effects of 6-MMPR are predominantly oral, and myelosuppression is infrequent. Luce et al (4) summarized the treatment of 20 patients with acute leukemia who were adequately treated with 6-MMPR; no remissions were observed. Bodey et al (5) reported that four of nine patients with AML achieved a CR when treated with the combination of 6-MP and 6-MMPR. These findings led to a larger study done by the Southwest Oncology Group.

MATERIALS AND METHODS

Patients ≥ 15 years of age who had not received 6-MP for > 14 days were accepted in the study. All patients were given a combination of 6-MP and 6-MMPR. The dose schedule was 300 mg/m²/day of 6-MP and 100 mg/m²/day of 6-MMPR given iv daily for 5 consecutive days and repeated in 14 days. If severe toxicity occurred, the dose was reduced by 33%. A patient not responding after three courses was considered a treatment failure. If a patient achieved a CR or partial remission (PR), he was given three consolidation courses at 2-week intervals at a maximum tolerated dose. Maintenance courses were given every 3–4 weeks.

RESULTS

Of 112 patients entered in the study, 95 were evaluable. There were 65 males and 47 females who ranged in age from 15 to 74 years (median, 35.5 years). Responses (CR + PR) were obtained in ten of 95 evaluable patients (10.5%). There were eight CRs (8.4%) and two PRs (2.1%). Three of ten patients (30.0%) with acute lymphocytic leukemia (ALL) responded (two CRs and one PR) while seven of 75 patients (9.3%) with AML responded (six CRs and one PR). There were no responses in ten patients with blastic cell crisis.

The durations of the CRs were > 460, 182, 150, 91, 78, and 77 days for the six patients with AML and > 512 and 210 days for the two patients with ALL. The two patients (one with AML and one with ALL) achieving the longest remissions were 19 and 20 years old.

Toxicity

Fifteen patients died within 12 days after the start of treatment and had no toxic effects. Of 80 remaining patients, 57 (71%) developed leukopenia and 36 (46%) developed thrombocytopenia. Stomatitis was reported in 47 patients (58%). Twelve patients had transaminase (SGOT) elevation which was transient in nine. Three patients died with hepatitis; autopsy findings showed acute hepatocellular degeneration in two of these patients.

DISCUSSION

Unfortunately, similar good responses were not seen in this larger group study using the identical dose regimen of Bodey et al (5). The CR rate in AML in this study was 16.3%, which is similar to that reported for 6-MP alone. Since 6-MP and 6-MMPR have different major toxic effects, they can both be used at optimal doses; however, further toxicity is seen with the addition of 6-MMPR, with frequently severe stomatitis occurring in more than one half of the patients. Thus, 6-MMPR does not improve the response rate in AML and does increase the toxicity. Hepatitis was noted in three patients who died and it is presumed, but not proven, that it was related to 6-MP since no other specific cause was found.

CONCLUSION

The combination of 6-MP and 6-MMPR does not offer better results than 6-MP alone in the treatment of adult acute leukemia. Its effect in ALL was not determined because the number of patients was too small and it was ineffective in blastic cell crisis.

REFERENCES

1. SCHABEL FM, JR, LASTER WR, JR, and SKIPPER HE. Chemotherapy of leukemia L1210 by 6-mercaptopurine (NSC-755) in combination with 6-methylthiopurine ribonucleoside (NSC-40774). Cancer Chemother Rep 51:111–124, 1967.
2. BENNETT LL, JR, BROCKMAN RW, SCHNEBLI HP, ET AL. Activity and mechanisms of action of 6-methylthiopurine ribonucleoside in cancer cells resistant to 6-mercaptopurine. Nature 205:1276–1279, 1965.
3. DAVIDSON JD, and WINTER TS. Purine nucleotide pyrophosphorylases in 6-mercaptopurine-sensitive and -resistant human leukemias. Cancer Res 24:261–267, 1964.
4. WRITING COMMITTEE: LUCE JK, FRENKEL EP, VIETTI TJ, ET AL. Clinical studies of 6-methylmercaptopurine riboside (NSC-40774) in acute leukemia. Cancer Chemother Rep 51:535–546, 1967.
5. BODEY GP, BRODOVSKY HS, ISASSI AA, ET AL. Studies of combination 6-mercaptopurine (NSC-755) and 6-methylmercaptopurine riboside (NSC-40774) in patients with acute leukemia and metastatic cancer. Cancer Chemother Rep 52:315–320, 1968.

James S. Hewlett*

Department of Hematology
and Medical Oncology
Cleveland Clinic
Cleveland, Ohio

Reprint requests to: Southwest Oncology Group, Operations Office, 3500 Rainbow Blvd, Suite 100, Kansas City, Kan 66103.

Gerald P. Bodey

Department of Developmental Therapeutics
University of Texas System Cancer Center
M. D. Anderson Hospital and Tumor Institute
Houston, Tex

Henry E. Wilson

Department of Hematology and Medical Oncology
Ohio State University Hospitals
Columbus, Ohio

W. J. Stuckey

Department of Medical Oncology
Tulane University School of Medicine
New Orleans, La

November 7, 1978

Combination Chemotherapy Pilot Studies in Head and Neck Squamous Cell Cancer[1]

We report here on our pilot studies with three separate drug combinations in previously treated head and neck cancer patients. Our objective was to identify a combination of drugs that might prove more effective than methotrexate alone. Failure to achieve a 50% tumor reduction for a minimum of 3 months in at least one of seven patients led to rejection of that treatment with a 95% confidence of its having a < 35% response rate. An expectation of at least a 35% response rate was necessary before considering a comparative trial with methotrexate.

MATERIALS AND METHODS

Twenty-three consecutive patients with incurable squamous cell carcinoma of the head and neck were selected for therapy. All patients had biopsy-proven carcinoma with measurable disease. Patients were evaluated every 3 weeks for response and toxic effects. A response was defined as a > 50% reduction in the sum of all measurable masses recorded as the longest diameter multiplied by its perpendicular diameter. The chemotherapy programs are displayed in table 1.

RESULTS

There were 15 males and eight females with an age range from 35 to 73 years. Two patients had a performance status of 0 to 1 (Zubrod classification) and 21 had a status of 2 to 3. All patients had received prior surgery and/or radiotherapy and all were stage IV; eight had received prior chemotherapy.

One of nine patients in the CMFB (cyclophosphamide, methotrexate, 5-fluorouracil, and bleomycin) group had a partial response for 2 months. There were no responders among seven patients receiving MLVP (methotrexate, citrovorum factor, vincristine, and *cis*-dichlorodiammineplatinum[II] [*cis*-platinum]). Two of seven patients receiving PB (*cis*-platinum and bleomycin) had partial responses lasting 2 weeks and 2 months, respectively. The response of the former patient was terminated by myelosuppression that resulted in infection and death. One patient who failed MLVP given in 3-week cycles subsequently responded to high-dose methotrexate-citrovorum factor alone given in a weekly schedule lasting for 5 months.

Three patients developed oral ulcerations and three had moderately severe vomiting from CMFB therapy. Myelotoxicity was not dose-limiting. A partially reversible decrease in creatinine clearance occurred in four patients receiving MLVP (50% decrease in one and 25%–50% decrease in three) and in one patient receiving BP (25% decrease). In both regimens, *cis*-platinum caused mild to moderate nausea and vomiting, which was more prolonged and less well-tolerated on the 3-day schedule.

DISCUSSION

Our pilot studies with these chemotherapeutic programs given to a selected population of patients with far-advanced disease have failed to suggest any increased effectiveness over that expected from single agents known to have activity in these tumors. Moreover, significant toxicity and one drug-related death occurred as a result of therapy. Consequently, none of these combinations at the doses and schedules employed are recommended for a randomized comparison to methotrexate.

One patient who progressed with a 3-week schedule of MLVP had tumor regression and control lasting for 5 months when treatment was switched to weekly high-dose methotrexate with citrovorum factor, confirming the impression of others that a weekly schedule of methotrexate is optimal (1). The observed stabilization of disease with weekly methotrexate and citrovorum factor in a patient who failed to respond to methotrexate and citrovorum factor combined with vincristine and *cis*-platinum on an every-3-week basis would suggest that drug combinations that compromise the frequency of administration of methotrexate may be less effective than methotrexate alone given in optimal scheduling.

[1]Supported in part by Public Health Service grant CA-22620 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

TABLE 1.—Chemotherapeutic regimens

Drugs	Dose and route of administration	Days of treatment	
CMFB			
Cyclophosphamide	100 mg/m², orally	Days 1–14	Repeat cycle every 28 days
Methotrexate	40 mg/m², iv push	Days 1 and 8	
5-Fluorouracil	600 mg/m², iv push	Days 1 and 8	
Bleomycin	7.5 mg/m², im	Days 2–6	
MLVP			
Methotrexate	500 mg/m²/24 hrs, iv infusion; dose escalation each subsequent cycle to toxicity	Day 1	Repeat cycle every 21 days
Citrovorum factor	40 mg q6hrs × 2 orally followed by 10 mg × 6 orally started 6 hrs after end of methotrexate infusion	Days 2–4	
Vincristine	1.0 mg/m² × 2 at 0 and 12 hrs; iv push	Day 1	
cis-Platinum	50 mg/m² 4-hr iv infusion	Day 8	
BP			
Bleomycin	15 mg/m², im	Days 3, 10, and 17	Repeat cycle every 21 days
cis-Platinum	30 mg/m², iv push, preceded by mannitol-furosemide-induced diuresis	Days 1–3	

REFERENCE

1. PITMAN SW, MILLER D, WEICHSELBAUM R, ET AL. Weekly high-dose methotrexate with leucovorin rescue as initial adjuvant therapy in advanced squamous cell carcinoma of the head and neck: a pilot study. *In* Adjuvant Therapy of Cancer (Salmon SE, and Jones SE, eds). Amsterdam, Elsevier/North Holland Bio-Medical Press, 1977, pp 467–473.

Adrian Bianco[2]
Samuel G. Taylor IV[3]
Steven Reich[4]
John M. Merrill
William D. DeWys[5]

Northwestern University
Medical School
Chicago, Ill 60611

November 7, 1978

[2]*Present address:* Little Company of Mary Hospital, Chicago, Ill.

[3]*Present address and reprint requests to:* Dr. Taylor at Rush-Presbyterian-St. Luke's Medical Center, Sheridan Road Pavilion, 6130 N Sheridan Rd, Chicago, Ill 60660.

[4]*Present address:* Bristol Meyer Laboratories, Syracuse, NY.

[5]*Present address:* National Cancer Institute, Bethesda, Md.

ANNOUNCEMENTS

Fourth Annual "Symposium on Advances in Cancer Treatment Research"

Baltimore, Maryland, April 19–21, 1979

The Baltimore Cancer Research Center, National Cancer Institute, and the Program of Continuing Education of the University of Maryland School of Medicine, are pleased to announce their 4th Annual "Symposium on Advances in Cancer Treatment Research," to be conducted in Baltimore, Maryland, April 19–21, 1979, at the Friendship International Hotel.

An experienced and internationally recognized faculty will participate. This Symposium is designed to update physicians and surgeons on some of the most recent developments in cancer treatment and current research including areas of clinical management, etiology, and cellular biology. Faculty members will be available throughout the Symposium to discuss any questions or interests of individual course registrants. A visit to the Baltimore Cancer Research Center can be scheduled for interested persons. The course is accredited for 16 hours of Category I credit for the PRA of the AMA by the Program of Continuing Education, University of Maryland School of Medicine.

For further information please contact: Jack Mason, PhD, Assistant Dean for Continuing Medical Education, 10 S Pine St, Rm 300E, Baltimore, Md 21201 (phone: 301-528-3956).

Seminars on Oncology at Roswell Park Memorial Institute

Buffalo, New York

There will be a seminar on "Immunotherapy of Cancer: Success or Failure" on May 10, 1979, organized by the Department of General Surgery at Roswell Park Memorial Institute. The chairperson is Dr. E. Douglas Holyoke.

There will be a $25.00 registration fee. *For further information contact:* Ms. Claudia Lee, Cancer Control, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

Second Advanced Course in Oncology

Boston, Massachusetts, May 7–11, 1979

The Boston University School of Medicine Cancer Research Center presents the Second Advanced Course in Oncology to be held on May 7–11, 1979, at the Copley Plaza Hotel, Copley Square, Boston, Massachusetts. This 5-day course will cover topics in medical, surgical, and hematologic oncology, and radiation medicine. Advances in clinical care and basic science will be stressed. The course is designed as a board review course for candidates who are preparing to take the National Boards in Oncology and is also suitable for established oncologists who would like to update their knowledge. A basic knowledge of oncology is assumed. The course director is Dr. Alexander S. D. Spiers.

The following topics will be covered: Surgical Oncology; Chemotherapy, Immunotherapy, and Tumor Cell Biology; Nutrition, Oncology Nursing, and Advances in Radiotherapy; Medical Oncology; and Hematologic Oncology, to be discussed Monday (May 7) through Friday (May 11), respectively.

A course banquet and Ladies' Social Program will be arranged. The fee is $300 for 5 days or $75 per day (Wednesday, $50). This course is AMA accredited in Category I for Physician's Recognition Award on an hour-for-hour basis. *For further information contact:* Robert M. Levin, MD, Boston University School of Medicine, Evans 113, 75 E Newton St, Boston, Mass 02118.

Psychosocial Care of the Dying Patient

A course entitled "Psychosocial Care of the Dying Patient" will be held in San Francisco on June 1–2, 1979. *For more information, write or call:* Extended Programs in Medical

Education, University of California, Rm 569 U, San Francisco, Calif 94143 (phone: 415-666-4251).

Fellowship in Medical Oncology

Applications are being accepted from candidates who are interested in a Joint Collaborative Training Program in Medical Oncology involving the major teaching hospitals of Brown University's Program in Medicine (Providence, Rhode Island), beginning July 1980 and July 1981. This Program offers clinical and research activities in clinical pharmacology and cancer chemotherapy, biochemical pharmacology, drug development, and tumor immunology, and participates in national cooperative chemotherapy groups to aid in the management of patients with solid tumors and hematologic malignancies seen at many of the hospitals in the Providence area. Prerequisites include 2 years of approved Medical Residency and A.B.I.M. eligibility. Candidates with academic interests are encouraged to apply. ***Send letter and curriculum vitae to:*** Paul Calabresi, MD, Professor and Chairman, Department of Medicine, or F. J. Cummings, MD, Head, Division of Medical Oncology, Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Ave, Providence, RI 02908.

*U.S. GOVERNMENT PRINTING OFFICE: 1979 281-225/10 1-3

If your address has changed and you wish to continue receiving this publication, please send your new address and the mailing label from this copy to

Cancer Treatment Reports
National Cancer Institute
8300 Colesville Rd
Blair Bldg, Rm 3A05
Silver Spring MD 20910
(301) 427-7322

If you do not wish to continue receiving this publication, please notify us at the address noted above and enclose the mailing label from this copy.

A publication of the National Cancer Institute

DHEW Publication (NIH) 79-22